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PRODUCTION OF RENAL INJURY IN THE WHITE RAT BY THE PROTEIN OF THE DIET

DEPENDENCE OF THE INJURY ON THE DURATION OF FEEDING, AND ON THE AMOUNT AND KIND OF PROTEIN *

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In 1919,[1] and again in 1923,[2] one of us (L. H. N.) reported the production of structural damage to the kidneys of rabbits by feeding them diets which contained large amounts of several kinds of protein. These experiments were adversely criticized on four scores: 1. The frequent spontaneous occurrence of renal lesions in the rabbit made it difficult to prove that any given injury was caused by the experimental procedure. 2. The herbivorous rabbit, habituated to a dietary low in protein, was a poor test object if the results are to be transferred to man. 3. The diets were inadequate. 4. The lesions were due to the acidity of the urine.

In reply to these criticisms, we began to feed properly constituted diets containing varying amounts and kinds of protein to white rats. During the ensuing interval of four years, a number of investigators have described the state of the kidneys of white rats which have eaten high protein diets. Some of these observers have noted well established lesions, whereas others failed to find satisfactory evidence of disease. We believe that we are able to explain this disagreement in the light of our own results. We will accordingly report our experiments and then deal with the publication of other workers.

EXPERIMENTAL WORK

We have carried out our feeding experiments according to the well known plan devised by Ferry.[3]

* From the Department of Internal Medicine, Medical School, University of Michigan.

* The expenses of this investigation were defrayed in part by grants from Parke, Davis & Co., and from the Fellowship Corporation.

1. Newburgh, L. H.: Production of Bright's Disease Through Feeding High Protein Diets, Arch. Int. Med. **24**:359 (Oct.) 1919.

2. Newburgh, L. H., and Clarkson, S.: Renal Injury Produced in Rabbits by Diets Containing Meat, Arch. Int. Med. **32**:850 (Dec.) 1923.

3. Ferry, Edna L.: J. Lab. & Clin. Med. **5**:735, 1920.

In order to supply adequate amounts of vitamins and salts, every rat, after being weaned, received daily 1 tablet containing 50 mg. of yeast extract. The diet itself contained 3 per cent of a tested cod liver oil and 4.5 per cent of the salt mixture described by Osborne and Mendel, when the protein was casein. Somewhat smaller amounts of the salt mixture were used when beef muscle or liver was the source of protein, because these materials contain considerable quantities of the various salts. The remaining ingredients of the diets were lard, cornstarch and protein. As the percentage of protein increased, the cornstarch was correspondingly decreased and finally omitted. In the case of diets high in protein, the percentage of lard was also decreased.

Diets Containing Casein

(1) About 7% Protein	Gm.
Casein	8.0
Lard	24.5
Corn starch	60.0
Salt mixture	4.5
Cod liver oil	3.0
	100.0

(2) About 10% Protein	Gm.
Casein	12.0
Lard	24.5
Corn starch	56.0
Salt mixture	4.5
Cod liver oil	3.0
	100.0

(3) About 15% Protein	Gm.
Casein	18.0
Lard	24.5
Corn starch	50.0
Salt mixture	4.5
Cod liver oil	3.0
	100.0

(4) About 20% Protein	Gm.
Casein	25.0
Lard	24.5
Corn starch	43.0
Salt mixture	4.5
Cod liver oil	3.0
	100.0

(5) About 26% Protein	Gm.
Casein	32.0
Lard	24.5
Corn starch	36.0
Salt mixture	4.5
Cod liver oil	3.0
	100.0

(6) About 32% Protein	Gm.
Casein	39.0
Lard	24.5
Corn starch	29.0
Salt mixture	4.5
Cod liver oil	3.0
	100.0

(7) About 61% Protein	Gm.
Casein	75.0
Lard	17.5
Salt	4.5
Cod liver oil	3.0

Salt Mixture

Calcium carbonate	134.8	Citric acid	111.1
Magnesium carbonate	24.2	Ferric citrate	6.34
Sodium carbonate	34.2	Potassium iodide	0.020
Potassium carbonate	141.3	Magnesium sulphate	0.079
Orthophosphoric acid	103.2	Sodium fluoride	0.248
Hydrochloric acid	53.4	Potassium aluminum sulphate	0.0245
Sulphuric acid	9.2		

The beef muscle proteins used in the diets were obtained from a manufacturer who stated that the material was made from fresh lean beef, mechanically freed of fat, rapidly dried and ground to a powder. Our analyses of samples showed that it contained 12.5 per cent nitrogen and about 10 per cent of ether soluble substances. It was assumed that all of the nitrogen was in the form of protein ($N \times 6.25$) and that 100 Gm. of the powder accordingly contains 78 Gm. of protein.

Since the beef furnishes a physiologic mixture of inorganic salts, the amount of the artificial mixture used was decreased as the percentage of beef in the diet was increased.

The third group of diets, the composition of which is given, was employed to study the effect of vegetable proteins on the kidneys. Since the seeds used contain at most 25 per cent of protein, this method of feeding vegetable proteins does not permit the construction of a

diet containing enough of these proteins to test the question in hand unless they are incorporated in a diet which also contains some other protein in concentrated form. Diets of the type commonly used by McCollum [4] were selected for this purpose, since his long experience has shown them to be adequate.

Diets Containing Beef Muscle Proteins

(8) About 12% Protein	Gm.
Beef	14.8
Lard	24.5
Corn starch	53.7
Salt mixture	4.0
Cod liver oil	3.0
	100.0

(9) About 17% Protein	Gm.
Beef	22.3
Lard	24.5
Corn starch	46.7
Salt mixture	3.5
Cod liver oil	3.0
	100.0

(10) About 24% Protein	Gm.
Beef	31.0
Lard	24.5
Corn starch	38.5
Salt mixture	3.1
Cod liver oil	3.0
	100.0

(11) About 31% Protein	Gm.
Beef	39.5
Lard	21.0
Corn starch	33.8
Salt mixture	2.7
Cod liver oil	3.0
	100.0

(12) About 38% Protein	Gm.
Beef	48.1
Lard	20.0
Corn starch	26.6
Salt mixture	2.3
Cod liver oil	3.0
	100.0

(13) About 72% Protein	Gm.
Beef	92.5
Lard	3.5
Salt mixture	1.0
Cod liver oil	3.0
	100.0

Diets Containing Vegetable Proteins

(14) About 18% Protein	Gm.
Wheat	30.0
Maize	22.0
Peas	20.0
Beans	20.0
Casein	3.5
Cod liver oil	2.0
Sodium chloride	1.0
Sodium carbonate	1.5
	100.0

(15) About 25% Protein	Gm.
Wheat	30.0
Maize	20.0
Peas	18.0
Beans	15.0
Casein	12.5
Cod liver oil	2.0
Sodium chloride	1.0
Sodium carbonate	1.5
	100.0

(16) About 32% Protein	Gm.
Wheat	20.0
Maize	20.5
Peas	20.0
Beans	15.0
Casein	20.0
Cod liver oil	2.0
Sodium chloride	1.0
Sodium carbonate	1.5
	100.0

(17) About 39% Protein	Gm.
Wheat	17.0
Maize	16.5
Peas	23.0
Beans	19.5
Casein	25.5
Cod liver oil	2.0
Sodium chloride	1.0
Sodium carbonate	1.5
	100.0

In order to obtain information in regard to the effect of nucleoprotein on the kidneys, a group of diets containing beef liver is being fed. Results from the following diet are available for comparison with those obtained from the foregoing diets. Fresh beef liver is minced, spread in a thin layer in broad pans, dried at about 70 C. and ground to a coarse powder.

4. McCollum and Simmons: Newer Knowledge of Nutrition, New York, The Macmillan Company, 1925.

The renal injury provoked by high protein diets might be caused by the extra work required of the kidneys in removing the unusually large amount of nitrogenous end-products. In order to increase the excretory work of the kidneys, urea in amounts sufficient to equal the nitrogen metabolism of a diet containing 40 per cent of protein was added to an otherwise normal diet. For this purpose, diet number 3 was used, and cornstarch was replaced by urea on the basis of the following calculations: A 40 per cent diet contains 6.4 per cent nitrogen and an 18 per cent casein diet, 2.88 per cent; therefore, 3.52 per cent nitrogen must be added. Urea contains 46.5 per cent nitrogen.

Accordingly, the inclusion of 7.57 Gm. of urea in 100 Gm. of a diet containing 18 per cent of casein will satisfy the requirements. The diet used is given as diet 19.

Diet Containing Beef Liver

(18) About 11% Total Nitrogen	
Liver	80.0 Gm.
Lard	16.0 Gm.
Salt mixture	1.0 Gm.
Cod liver oil	3.0 Gm.
	100.0 Gm.

Diet with Urea Substituted for Cornstarch

(19)	
Urea	7.57 Gm.
Casein	18.00 Gm.
Lard	21.00 Gm.
Corn starch	43.00 Gm.
Salt mixture	4.50 Gm.
Cod liver oil	3.00 Gm.
	100.07 Gm.

We have used two standard methods of obtaining evidence in regard to the state of the kidneys; namely, urinalysis and histologic examination. In an earlier paper, we described a simple apparatus [5] for obtaining satisfactory samples of urine.

Both the experience in the clinic and that of laboratory workers, who have examined the urine of animals, has taught us that urine which contains neither albumin nor casts is coming from normal kidneys, and that the presence of much albumin and especially the occurrence of numerous casts is a reliable sign of disease of the kidneys. This latter statement, however, should be modified by noting several exceptions. Albuminuria without casts is found in human beings in the form of orthostatic albuminuria, and transient albuminuria may be

5. Curtis, A. C., and Newburgh, L. H.: Toxic Action of Cystine on the Kidney, Arch. Int. Med. **39**:817 (June) 1927.

produced by violent exercise. In experimental animals, the acidified urine often becomes opalescent or even cloudy on being boiled. Those exceptional cases of albuminuria do not indicate disease of the kidneys, and accordingly makes the detection of albumin a less reliable sign of disease of the kidneys than the observation of casts. The literature, however, does not contain any account of the occurrence of heavy albuminuria in the urine of laboratory animals the kidneys of which were free from disease. After diligent search, a few investigators have noted one or two casts in the urinary sediment of laboratory animals the kidneys of which were assumed to be normal. The easy detection of casts, however, is accepted as a reliable sign of renal abnormality.

TABLE 1.—*The Effect of Time and of the Amount of Protein on the Occurrence of Casts* *

Per Cent of Protein	Days 60	120	180	300	360	420	480
8	V 0	V 0	V 0	.. ..	IV 0	.. ..	.. ..
12	X 10	V 0	V 0	IX 0	VIII 12†	I 0	V 20
18	XVII 0	XIX 5	XV 7	XXI 9‡	XVIII 17‡	XI 27	VIII 12
25	XV 6	XXII 23	XXIII 70	XXII 57	XVII 53	X 50	VII 86
32	XII 0	XVII 41	XXIV 79	XVIII 66	XXII 91	XII 100	X 100
39	XII 17	XXVII 55	XXV 92	XX 100	XX 100	XIII 100	VII 100
75	VIII 100	VIII 100	VIII 100	VIII 100	.. ...	VI 100	.. ..

* The Roman numerals indicate the number of animals the urine of which was examined. The Arabic numerals give the per cent of urines in which one or more casts were found.

† The one animal of the group, the urine of which contained casts, also had hematuria. At autopsy, infarction of the left kidney was found.

‡ One of the two animals of the group, the urines of which contained casts, was obviously ill. It was found dead a few days later.

‡ One of the three animals of the group, the urine of which contained casts, had an acute infection from which it died three hours after the urine was obtained.

CORRELATION BETWEEN AMOUNT OF PROTEIN AND EXTENT OF RENAL INJURY

With this background in mind, we undertook routine examination of the urine of the animals for the presence of albumin and of casts. We hoped thus to be able to answer in a broad way two questions: 1. Is a high protein diet injurious to the kidneys of the rat? 2. If injurious, what is the largest amount of protein that may be habitually eaten without harming the rat's kidneys?

In table 1, we have brought together our observations in 565 twenty-four hour specimens. The question of albuminuria is omitted for the sake of simplicity. The animals are grouped according to the amount of protein contained in the diet, regardless of the kind. The

table shows the number of animals from which specimens were obtained and how many of them contained casts. The urines were, as a rule, examined every second month. The animals used for this study were those that received diets numbers 1 to 17, inclusive. Thus, in the first table, protein is regarded as a single factor regardless of its type. The data contained in the table lead clearly to several conclusions: The percentage of animals, the urine of which contains casts, increases as the protein of the diet reaches the higher concentrations. Diets the protein content of which is 18 per cent or less exhibit only a minor and an irregular tendency to cause cylindruria. The factor of time is marked as an agency which increases the percentage of urines containing casts when the protein of the diet is 25 per cent or more, but appears to have only a trivial and irregular effect when the dietary protein is low.

The information contained in table 1 possesses an inherent weakness since it lacks the means of forming any opinion about the degree of

TABLE 2.—*Relation between the Amount of Dietary Protein and the Number of Casts and Per Cent of Albumin in the Urine*

Per Cent of Dietary Protein	240 Days			480 Days		
	Animals, Number	Albumin, per Cent	Casts, Number	Animals, Number	Albumin, per Cent	Casts, Number
12	3	0	0	5	0.2	4
18	11	0	3	10	0	4
25	8	0	0	6	0.6	83
32	7	0	44	12	0.8	351
39	9	0.2	52	10	0.4	652
75	15	0.3	1,412	4*	1.4	4,705

* Only two animals in this group were alive at 480 days. The averages recorded in the table were obtained from counts made on the 410th day.

injury caused by the protein. The difference between urines recorded as normal and abnormal might be only one cast, in which case the injury would be negligible; or, 1,000 casts would be indicative of a significant amount of injury. In order to overcome this difficulty, the casts have been counted in a considerable number of urines by the method described by Addis.[6] The percentage of protein in the same specimens has been determined by the method of Folin.[7]

Table 2 shows the average number of casts and the average amount of albumin in the twenty-four hour samples of urine in relation to the concentration of protein in the diet and the duration of the feeding. When the number of casts is used as a measure of renal injury, it becomes evident that diets containing 12 and 18 per cent of protein are harmless. Twenty-five per cent of protein causes a small injury if the diet is continued more than a year. Thirty-two and thirty-nine per

6. Addis, T.: Clinical Classification of Bright's Diseases, J. A. M. A. **85:** 163 (July 18) 1925.

7. Folin: J. Biol. Chem. **18:**283, 1914.

cent of protein cause the appearance of an abnormally large number of casts in eight months, and the injury becomes slowly more marked during the second eight months. When the diet contains 75 per cent of protein, the injury is well established in eight months and increases greatly during the subsequent months.

CORRELATION BETWEEN KIND OF PROTEIN AND EXTENT OF RENAL INJURY

The data dealt with thus far show clearly that the degree of damage to the kidney (measured by the urinary observations) is determined by the amount of protein in the diet for similar intervals of time. We wish now to bring forth evidence intended to show how greatly the degree of nephropathy depends on the kind of protein ingested.

TABLE 3.—*Relation between the Type of Protein and the Degree of Renal Injury when the Protein is One Third of the Diet*

	240 Days		450 Days	
	Albumin, per Cent	Casts, Number	Albumin, per Cent	Casts, Number
Casein	0.1	37	0.1	312
Beef muscle	0.3	60	1.1	1,444

TABLE 4.—*Relation between the Type of Protein and the Degree of Renal Injury when the Protein is Three Fourths of the Diet*

	240 Days		450 Days	
	Albumin, per Cent	Casts, Number	Albumin, per Cent	Casts, Number
Casein	0.2	783	0.5	992
Beef muscle	0.5	2,130	1.5	5,900
Liver	3.0	8,900		

Table 3 gives the average per cent of albumin and the number of casts in twenty-four hour specimens of urine for groups of rats, one third of whose diet consisted either of casein or of lean beef muscle.

Table 4 is constructed in the same way to compare casein, beef muscle and liver when approximately three fourths of the diet is protein.

These two tables bring out the fact that the nature of the protein fed is at least as important a factor as the amount of protein in both the production of injury and in the degree of injury. This statement derives further support from the course of events in the case of the rats that received the liver diet. These five animals, all of which showed the heavy albuminuria and the great numbers of casts, were unable to survive the diet a year. Four of them lived eight months and the fifth one, eleven and one-half months. Their early failure seemed the more significant, since they grew more rapidly and reached a greater weight than any other group studied.

The importance of taking the nature of the protein into account is likewise strongly emphasized by a comparison of the histologic changes caused by the different proteins.[8]

Thus, the only injury obtained by us with casein was restricted to the tubules. In animals that received diets containing 75 per cent of casein for more than a year, the tubules contained numerous casts and there was moderate degeneration of the epithelium of the convoluted tubules and some tubular dilatation.

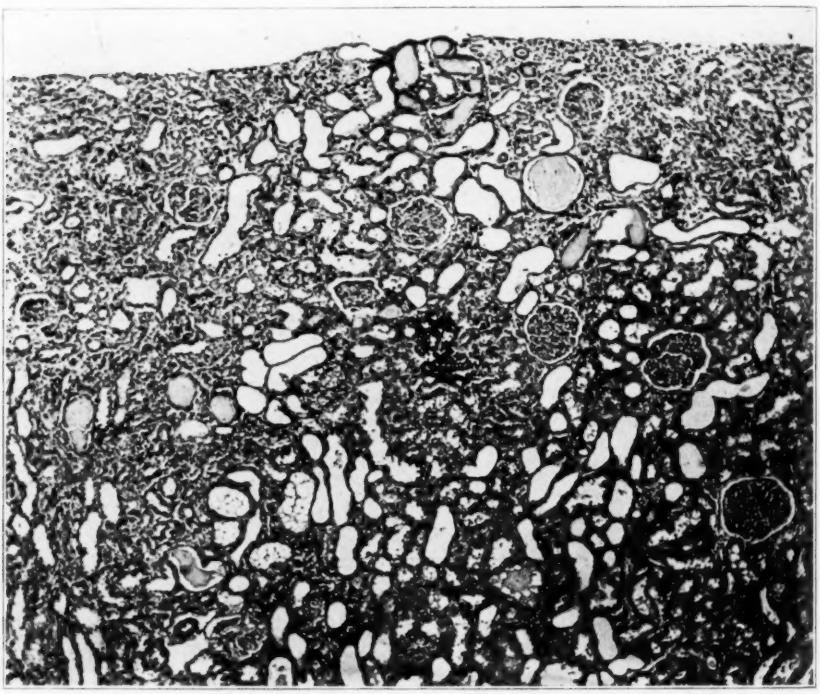

Fig. 1.—Diffuse tubular dilatation and atrophy, and many casts. This rat ate a diet containing 31 per cent of beef muscle proteins for fifteen months.

Beef muscle proteins, in addition to causing a more severe tubular injury, are capable of injuring the glomeruli and of causing a patchy fibrosis. Figure 1, which is a photomicrograph from a rat that had

8. For microscopic study, we used only kidneys from animals that were killed by a blow on the head. Immediate autopsy was performed, and the tissues were hurried into formaldehyde solution; thus, any possible confusion due to postmortem change was avoided. The standard technic for paraffin sections was followed. The sections were stained with hematoxylin and eosin.

eaten a diet containing 31 per cent of beef proteins for fifteen months, showed more tubular injury than was found in animals that had taken the diet containing 75 per cent of casein for the same length of time. Figure 2 shows glomerular damage from 31 per cent of beef muscle proteins in eighteen months. Definite glomerular lesions were not seen in any of the rats the diet of which contained less than 31 per cent of protein nor in any of the casein series, including even the rats that took diets containing 75 per cent of casein. When the rats live on diets containing 38 per cent of beef muscle proteins for more than one year, a

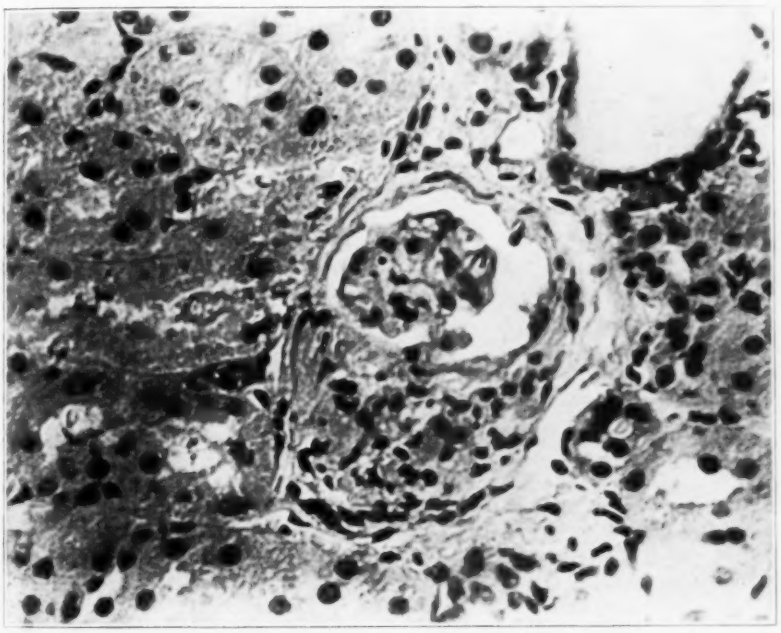

Fig. 2.—Marked thickening of Bowman's capsule; from a rat that took the diet containing 31 per cent beef muscle proteins for eighteen months.

third sign of renal injury appears. Figure 3 is from an animal that was killed after thirteen months of such a diet. The photomicrograph shows one of a number of small patches of fibrosis in addition to glomerular and tubular abnormality. The kidneys of old rats frequently show scattered minute areas of fibrosis. Osborne, Mendel, Park and Winternitz called attention to this increase in connective tissue in rats more than 500 days old, but that were otherwise presumably normal. Figure 3 is from an animal 420 days old. The fibrotic areas seen in sections of kidneys from rats that had received the diet containing 38 per

cent of beef muscle protein for from 420 to 450 days, were much larger and more numerous than those found by us in some of the rats of equal age, the diets of which contained 18 per cent of casein or beef muscle protein. Diets containing 72 per cent of beef protein are capable of causing a considerable degree of fibrosis. Figure 4 shows one of a number of such patches. This rat had been on the high beef diet 470 days when he was killed. The kidneys of the large number of rats that had received diets containing 18 per cent of protein or less were, in general, free of fibrotic areas. In a few instances, occasional minute patches

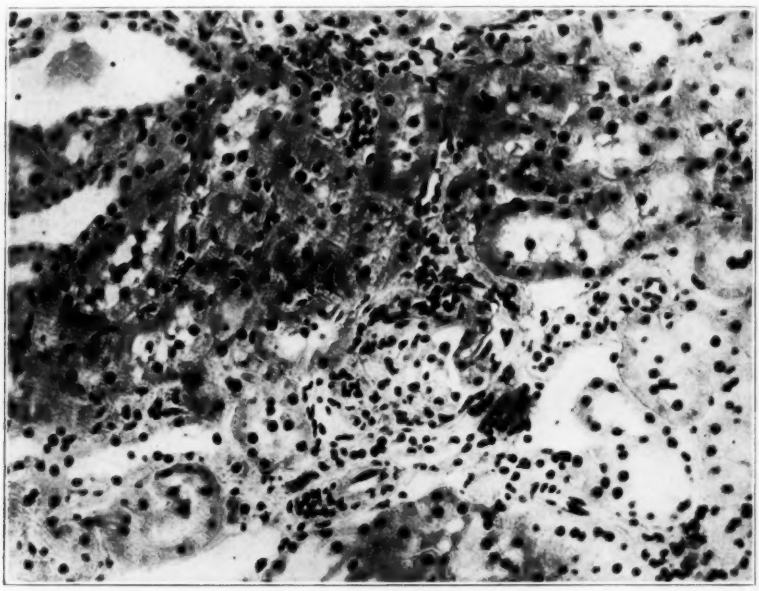

Fig. 3.—A small patch of fibrous tissue partially obliterating the glomerulus. This rat received a diet containing 38 per cent of beef muscle proteins for fourteen months (420 days).

of cellular increase were noted; they were never large enough to occupy more than a portion of a high power field, and never caused thickening of Bowman's capsule or injury of the tubules separated by the new cells. The area reproduced in figure 4 is one of many found in the rats that had taken the 72 per cent beef protein diet for more than one year. The large size of this area contrasts sharply with the smallness of the foci seen in the low protein animals; there is at least a ten-fold difference. Figure 5 shows in more detail, the partial fibrosis of the glomerulus and the destruction of the tubules in one part of a fibrotic patch.

None of the casein or beef diets caused changes sufficiently great to definitely alter the gross appearance[9] of the kidneys. However, when the diet contains 75 per cent of beef liver, the appearance of the kidneys is strikingly altered. In so short a time as 300 days, the outer surface of the kidneys had everywhere become markedly granular and the color had changed from the normal red-brown to gray-yellow, mottled with spots of purple. Figure 6 is a photograph of a normal kidney and figure 7, the kidney of a rat that was killed after it had eaten the liver diet for ten months. The granular character of the latter is evident. Figures 8, 9, 10 and 11 are microscopic views of the

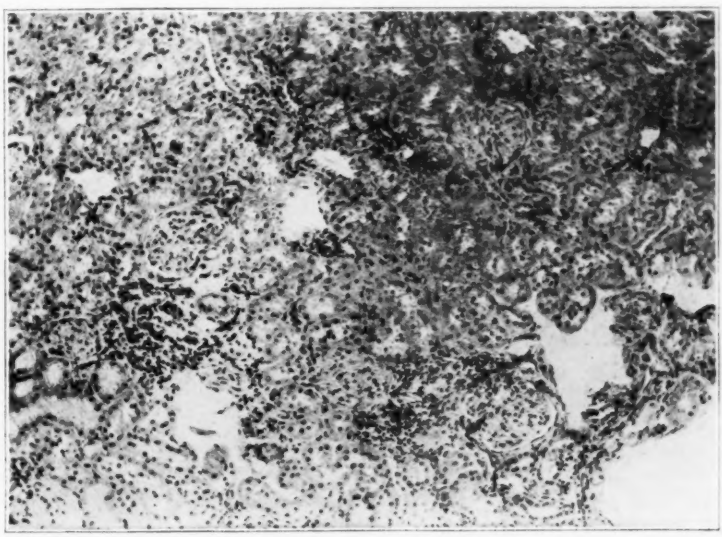

Fig. 4.—Area of patchy fibrosis concentrated about the glomeruli. From a rat that ate a diet containing 72 per cent of beef muscle proteins for sixteen months.

9. The kidneys of rats on the higher levels of protein were larger than those of animals the protein intake of which was less. Averages from four diets are contained in the accompanying table.

Our observations agree with those of earlier observers who pointed out that the weight of the kidney is related to the amount of protein in the diet. Moise and Smith (Arch. Path. **4**:530 [Oct.] 1927) published the most recent work dealing with the character of this increase in weight.

Per Cent of Protein	Kidney Weight, Gm.	Kidney Weight / Body Weight
18	1.7	0.82
25	1.7	0.80
32	2.4	0.89
39	2.5	0.99

kidney of a rat that was killed when he had taken the liver diet for 350 days. The great increase in connective tissue, especially in the labyrinths, which causes contraction of the kidney in these areas, is well seen in figures 8 and 9. The marked cystic dilatation of some of the tubules, a well-known feature in chronic nephritis of man, is likewise clear. Various forms of glomerular injury may also be seen: namely, great thickening of Bowman's capsule by layers of connective tissue, adhesions between the capillary tufts and the capsule, lobulation of the glomeruli and fibrosis of some tufts.

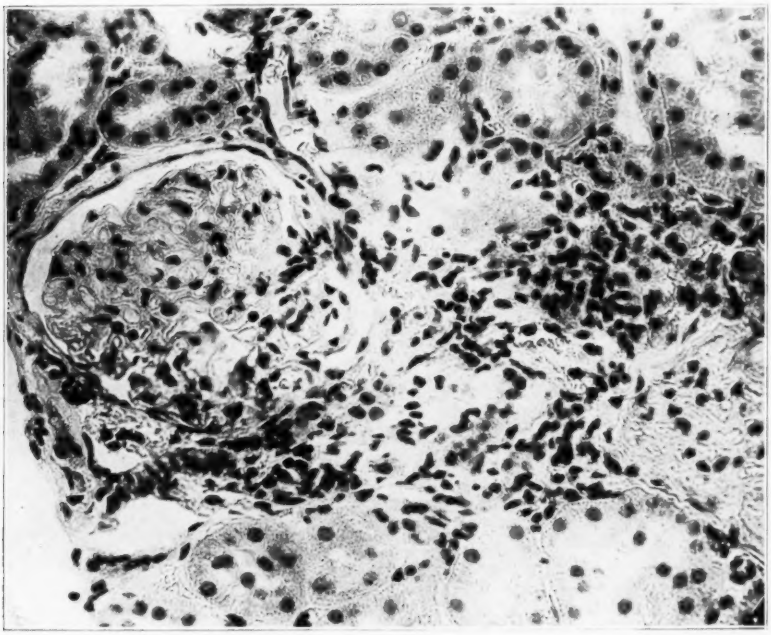

Fig. 5.—Partial fibrosis of a portion of the glomerulus and the destruction of the tubules in one part of a fibrotic patch. Occasional red blood cells are seen in the area. This is part of the field shown in figure 4.

The renal lesions that have been described are not due to a dietary deficiency, for the diets that caused the most injury also permitted the best growth. Inspection of figure 12 will show that the groups of rats that ate the high beef diets and the liver diet, grew more rapidly than did the animals on which Donaldson's standard growth curve is based. On the other hand, the animals the diets of which were too low in protein to permit growth at the normal rate had normal kidneys.

REVIEW OF LITERATURE

The foregoing experiments have convinced us that the injuries to the kidney described were caused by the protein of the diet and that the degree of injury depends on the concentration of the protein, its type and the duration of the feeding. While we were carrying on our experiments, a number of papers dealing with this subject appeared.

Fig. 6.—Normal kidney showing the smooth surface and the mottling due to the stellate veins.

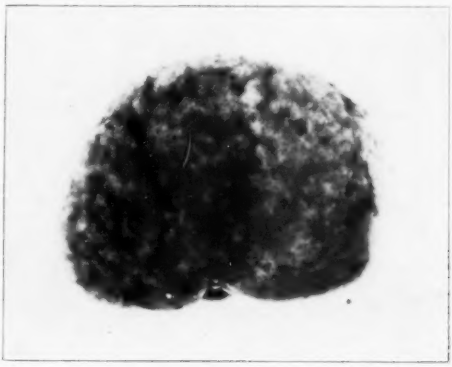

Fig. 7.—Kidney from a rat that ate a diet containing 75 per cent of dried beef liver for ten months; the same enlargement as figure 6. The surface is everywhere coarsely and finely granular, and the normal venous markings have disappeared.

Some observers reported results similar to ours, while others did not see any evidence of the deleterious effect of protein on the kidneys. The three papers of Drummond, Crowden and Hill,[10] Reader and

10. Drummond, J. C.; Crowden, G. P., and Hill, E. L. G.: J. Physiol. **56**: 413, 1922.

Drummond,[11] and of Kennedy [12] may be dealt with together. The primary interest in each is the effect of a high protein diet on reproduction and growth. A diet containing about 75 per cent of casein was used to test the influence of an excess of protein. The rats were killed after they had been on the diet four months. The urine was not examined. The kidneys did not show any microscopic abnormalities. This outcome convinced these authors that high protein diets do not injure the kidneys of rats. This generalization is not warranted by the data in their

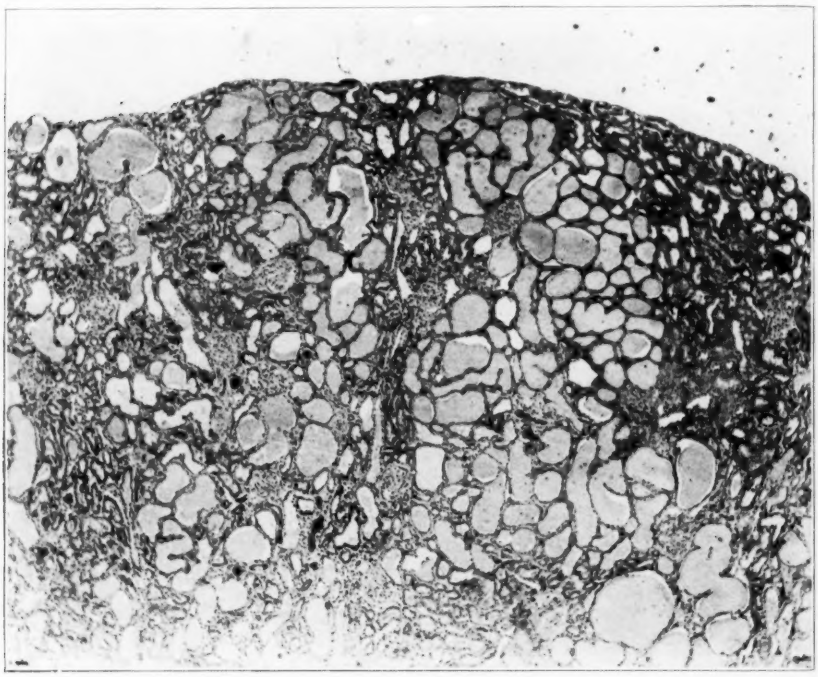

Fig. 8.—A low power view of a section of kidney from a rat that took a diet containing 75 per cent of dried beef liver for twelve months. Showing the bands of contraction most marked in the labyrinths, and the general dilatation of tubules in the intermediate areas.

possession. They were justified merely in stating that a high casein diet did not produce histologic changes in the kidneys when the animals were fed for the brief period of four months. Had they published such a statement, they would have found themselves in entire agreement

11. Reader, V. B., and Drummond, J. C.: J. Physiol. **59**:472, 1925.
12. Kennedy, W. P.: Quart. J. Exper. Physiol. **16**:281, 1926.

with us. Casein, as we have already pointed out, produces only a small amount of injury and must be fed in large amounts for a least one year in order to obtain microscopic evidence of damage.

Addis, MacKay and MacKay [13] fed a group of rats a diet containing 74 per cent of casein for 330 days. They considered the kidneys histologically normal and found no more casts in the urine of the animals on the high diet than in that of the controls. A careful comparison of their method of examining the urine with ours suggests why they did not find an abnormally large number of casts in the case of the rats fed

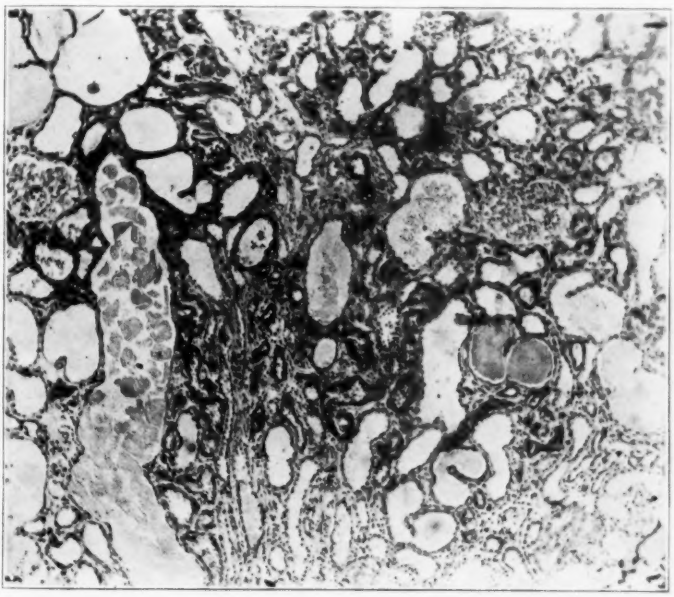

Fig. 9.—A higher power view from the same kidney as figure 8, to show a band of contraction in which glomeruli are not visible. The dilated and atrophied tubules are widely separated by the connective tissue.

on a diet high in casein. We collected urine for twenty-four hours, centrifugalized the whole specimen and pipetted off the supernatant fluid down to 0.1 cc. All the casts voided in twenty-four hours were thus collected in 0.1 cc. A drop of this mixed urine was placed on a counting chamber and 1 c.mm. counted. We found, on the average, eight casts in 1 c.mm. in the urine thus prepared from rats that had received 75

13. Addis, T.: MacKay, E. M., and MacKay, L. L.: J. Biol. Chem. **71**:139, 1926.

per cent of casein for ten months. Addis and his co-workers, on the other hand, obtained fresh urine by causing the rat to inhale a whiff of ether. They stated that a specimen so obtained has one-tenth the volume of the twenty-four hour amount. If their rats voided casts at the same rate as ours, their fresh specimen would contain eighty casts. Since their method of counting is the one used by us, they should have found less than one cast in the 1 c.mm. counted. We believe that this difference in procedure is the basis for their statement that "An occasional cast

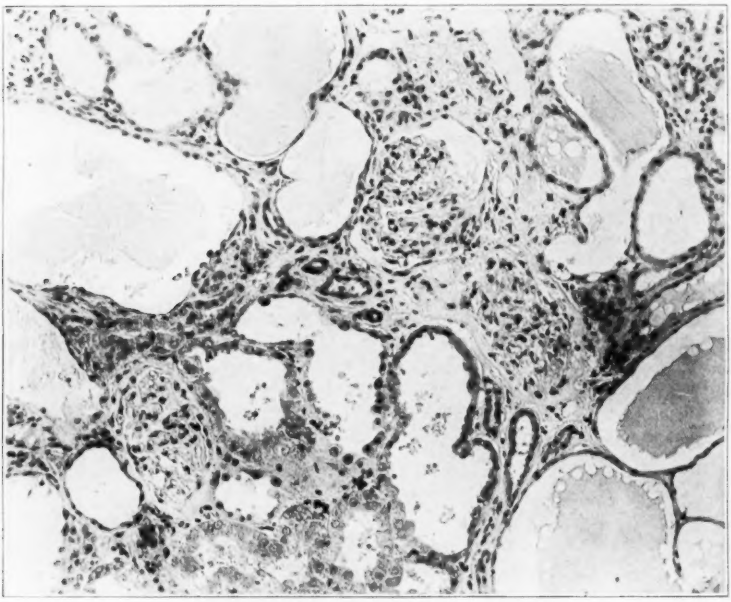

Fig. 10.—A still higher magnification of the section presented in figure 9, to show the thickening of Bowman's capsules with extension of the connective tissue into the capillary tufts, and the cystic dilatation of tubules the lining of which resembles pavement epithelium.

was found at one time or another in the urine collected from nearly all of the experiments. They were no more frequent in the high protein than in the control groups."

Jackson and Riggs [14] likewise studied the effect of a diet containing 76 per cent of casein on the kidneys of rats. In regard to urinalysis they state:

14. Jackson, H., Jr., and Riggs, M. D.: J. Biol. Chem. **67**:101, 1926.

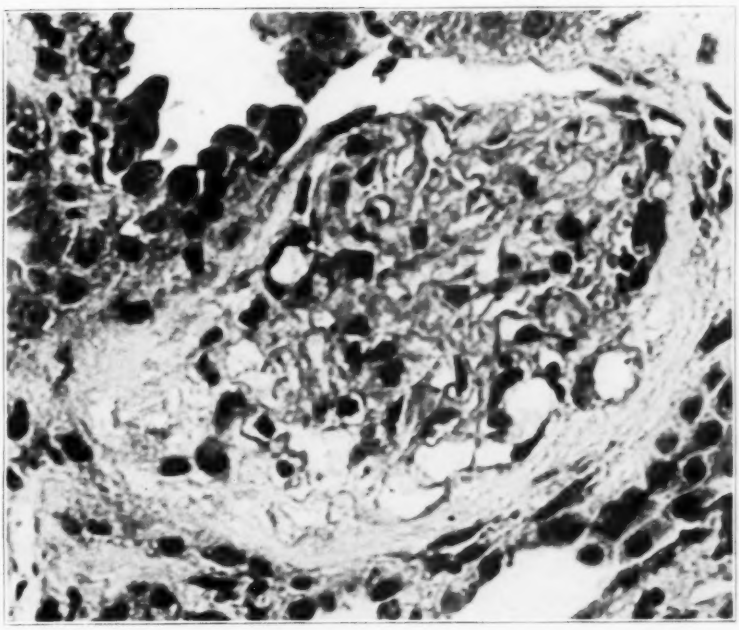

Fig. 11.—High power magnification of a glomerulus from the same kidney to show hyaline thickening of the capsule, adhesions of the tuft to the capsule and the partial obliteration of the capillary tuft.

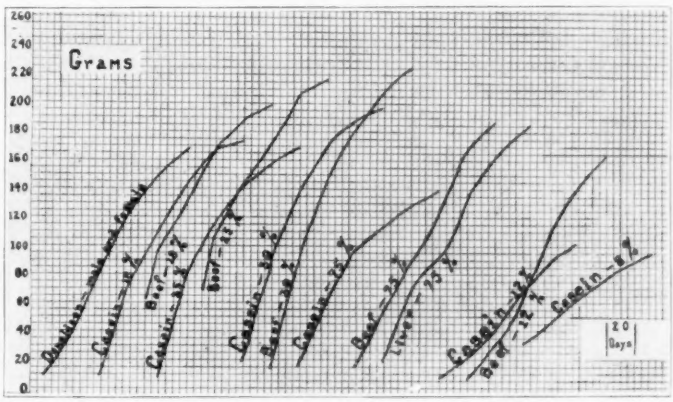

Fig. 12.—Growth curves. The diets which caused the greatest renal damage were adequate for normal growth.

During the progress of the experiment, each rat was periodically placed in a metabolism cage and urine collected. Normal rats do not show casts or red cells. Casts were not found in any of the specimens except rarely in one of the rats on high protein plus alkali.

In the absence of any detailed description of the method for obtaining specimens, we are left with the question whether casts would not have been found in the urines of the rats on the high casein diets, if more care had been taken in the collection and preservation of the specimens. These investigators were also unable to detect any histologic abnormalities in the kidneys from rats that had eaten the excessive amounts of protein for more than one year.

Jackson and Riggs also fed two rats a diet containing 20 per cent of casein and 56 per cent of egg albumin, with negative results. This diet failed to cause hypertrophy of the kidneys—a change which was caused by their high casein diet and the response, to a variety of high protein diets, obtained by all observers who have weighed the kidneys. Furthermore, the daily urinary nitrogen from the two rats receiving egg albumin was 0.95 Gm., whereas the average from the high casein group was 1.21 Gm. This difference corresponds with the differences in ingested nitrogen. The casein group took 0.631 Gm. per hundred grams of body weight, but the rats that received egg albumin took only 0.525 Gm. nitrogen per hundred grams. The less intense nitrogen metabolism in these latter rats may, in part, account for the absence of signs of injury.

Osborne, Mendel, Park and Winternitz [15] likewise stated, in several preliminary reports, that high casein diets did not injure the kidneys of rats. Only five of the large number of rats fed the casein diets by them were kept on the diet more than 300 days, and in no case did the experiment last 350 days. When the positive results, reported by Polvogt, McCollum and Simmonds,[16] became known to them, they noted that these workers had used a different type of diet and had fed their animals much longer. They accordingly fed a similar diet, the composition of which is as follows, to six rats, for 400 days or longer: Wheat, 30 Gm.; corn, 20 Gm.; casein, 20 Gm.; liver, 20 Gm.; navy beans, 7.5 Gm.; calcium carbonate, 1.5 Gm., and sodium chloride, 1 Gm.

Furthermore, to control the old age factor, they secured a group of rats, 500 days, or more, of age. These animals had been fed a stock diet containing the usual moderate concentration of protein.

From the detailed account of the histologic study of the kidneys of 200 rats, ninety-two of which had been on diets rich in protein from

15. Osborne, T. B.; Mendel, L. B.; Park, E. A., and Winternitz, M. C.: Am. J. Physiol. **72**:222, 1925.

16. Polvogt, L. M.; McCollum, E. V., and Simmonds, N.: Bull. Johns Hopkins Hosp. **34**:168, 1923.

seven to 400 days and 108 of which were controls, we consider the following sentences significant:[17]

> In many rats on the very rich protein diets, the dilatation of the tubules was marked enough to enable us to guess correctly at a glance the high protein nature of the feeding. Dilatation of the tubules was not present or, at least not clearly recognizable, in the sections of other rats which were either on the high protein diets for short periods or else on diets in which the proportion of protein was not particularly large. . . . . Some rats on the high protein diets showed actual lesions. In certain of the control animals, lesions of an exactly similar nature were found. . . . Eighteen rats on the high protein diets showed these lesions, the remaining eighteen, on very high protein diets, did not. The lesions were severe in five of the six rats that had been on the Polvogt, McCollum and Simmonds diet 400 days. Among the control rats, the kidneys of only five were affected. All were 515 days old, or older. The lesions were of the same type as those seen in the Polvogt, McCollum and Simmonds diet.

It is conceded by Osborne, Mendel, Park and Winternitz that the dilatation is caused by the diets rich in protein, and that animals on such diets develop focal lesions much more frequently and earlier than the controls. But these authors appear to be in doubt in regard to the significance of these histologic departures from the normal. We interpret them as evidence of the harmful effect of high protein diets; and especially so, since the severe focal lesions were found in the group of rats that received a diet containing 20 per cent of liver.

If this latter group is left out of consideration it will be realized that the views expressed by the writers of the papers discussed thus far were derived from their experience with casein. From what has already been said about casein, it is not difficult to understand how they reached the conclusion that high protein diets are not harmful to the kidneys. But our own experiments show clearly that it is not permissible to generalize about protein as if it were an entity. The most that can be learned from the experiments of these writers, if their work is accepted without any deduction, is that diets rich in casein do not injure the kidney. But the information obtained about casein must not be transferred to protein in general. A sharp distinction must be made between the phrases "high casein diets" and "high protein diets," for we have shown that certain other proteins produce an unmistakable nephropathy.

It is for that reason, we believe, that both Evans and Risley[18] and Polvogt, McCollum and Simmonds obtained injury to the kidney from the diets used by them. The composition of the diet used by the latter workers has already been given. Their rats were killed when they had eaten the food 400 days. In summarizing their experiments, they state:

17. Osborne, T. B.; Mendel, L. B.; Park, E. A., and Winternitz, M. C.: J. Biol. Chem. **71**:317, 1927.

18. Evans, N., and Risley, E. H.: California & West. Med. **23**:437, 1925.

"All the rats fed the diets high in protein had lesions of the kidneys of considerable severity. On the other hand, animals of middle age from the breeding stock, all had essentially normal kidneys." Evans and Risley used proteins from several sources including meat, peanuts, soy beans and wheat gluten. These investigators took the precaution to send the animals to the pathologic laboratory, identified by number alone, so that the one who made the histologic examination of the kidneys did not have any knowledge of the diet the animal had received. They found that animals fed on high protein rations for prolonged periods showed nephritic changes without exception.

Finally, Moise and Smith [19] accentuated any effect of diet by unilateral nephrectomy. Such animals were fed either an 18 per cent or an 85 per cent casein diet. They did not observe any significant anatomic evidence of renal injury in the animals on the "standard" diet. In animals that had been on high casein ration for 90, 120 and 150 days after nephrectomy, however, "significant" glomerular and tubular changes were found without exception. The lesions were not merely isolated focal changes but were conspicuous and relatively widespread. They included proliferation of the epithelium of Bowman's capsule, adhesions between the tuft and capsule, fibrous thickening of the capsule, partial fibrosis of the tuft, degenerative changes in the tubular epithelium and areas showing an increase in the interstitial tissue They also found casts and a markedly increased amount of albumin in the urines of rats on the high casein diet, after eighty-eight days. These experiments show that severe kidney disease may be caused in a relatively short period of time by a protein even as mildly irritating as casein, when its effect is borne by only one kidney.

The renal injury that is caused by diets containing an excess of protein might be brought into existence in many ways. Addis [13] has shown that it is not due to a change in the hydrogen ion concentration of the urine. One of us (L. H. N.) [1] was unable to find any of the dietary protein in the blood or urine. No one has demonstrated the presence of toxins in the systemic circulation, resulting from bacterial decomposition of the protein in the intestines. The addition of large amounts of urea to an otherwise normal diet to increase the excretory work of the kidneys failed to cause hypertrophy in the hands of Osborne, Mendel, Park and Winternitz, Hinman,[20] or Addis, MacKay and MacKay.

COMMENT

Our own urea diet number 19, which caused the rat to excrete an amount of urinary nitrogen equivalent to that yielded by a diet containing 40 per cent of protein, was fed to a group of rats for one year. In

19. Moise, T. S., and Smith, A. H.: Effect of High Protein Diet on Kidneys, Arch. Path. **4**:530 (Oct.) 1927.

20. Hinman, F.: J. Urol. **9**:289, 1923.

no case did we obtain evidence from the urine, or by microscopic examination of the kidneys, that the prolonged excretion of this large amount of nitrogen was harmful to the kidneys; nor did this diet in which the urea content was high cause any enlargement of the kidneys. The increase in size caused by high protein diets is apparently something more than a response to increased activity. It appears to have a more specific cause.

Positive evidence dealing with the mechanism of the protein injury has been obtained by studying the effect of the amino-acids. Our injection [21] and feeding experiments [5] have shown that some of the amino-acids resulting from the digestion of protein are nephrotoxic. We obtained the most striking evidence of injury from the injection of cystine and of tryptophan. Casein is low in these amino-acids, whereas muscle protein is relatively rich in both of them. We believe that these differences in the amino-acid make-up of these two proteins is, at present, the best explanation for the difference in the degree of nephrotoxicity shown by them.

SUMMARY

The effect of diets containing several different proteins in varying concentrations on the kidneys of the white rat has been studied. Casein, beef muscle, beef liver and the seeds of cereals and legumes were used as the sources of the proteins.

It was found that the degree of injury was determined by the type of protein, its concentration and the length of the feeding.

The character of the protein is more important than either of the two other factors.

Diets containing 75 per cent of dried liver produce a granular kidney in less than one year, but the same amount of casein, fed sixteen months, causes only a moderate tubular injury. The effect of a similar amount of beef muscle is intermediate between these two. The surface of the kidney remains smooth, but microscopic sections show numerous areas in which seriously injured glomeruli and necrotic tubules are embedded in fibrotic tissue.

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21. Newburgh, L. H., and Marsh, P. L.: Renal Injuries by Amino-acids, Arch. Int. Med. **36**:682 (Nov.) 1925.

GENERALIZED GRANULOMATOUS LYMPHADENITIS ASSOCIATED WITH DIFFUSE PROGRESSIVE FIBROSIS OF THE LUNGS*

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BOSTON

It is thought that the following case should be recorded because of the apparently unique combination of lesions which were severe enough to cause death. It needs little comment. It does not resemble any other disease entity which I have been able to find, but, on the contrary, the clinical signs and symptoms are so clearcut that it seems to present an entity in itself. The clinical course and pathologic observations do not correspond with any clinical or pathologic condition within the knowledge of a considerable number of clinicians and pathologists who have seen the case. But because of these clearcut features, a similar case in the future might be recognized in time to permit a more complex study of the condition. I have been unable to find anything remotely resembling it in the literature. As complete a description as possible is therefore given, only impertinent details being omitted.

REPORT OF CASE

Clinical History.—A boy, aged 16, entered the Montreal General Hospital, March 2, 1927, in the service of Dr. C. P. Howard, complaining of a lump in the left groin, slight cough and vague pain over the pit of the stomach. He had one brother and five sisters, who were alive and well; his parents were also well. With the exception of the common childhood infections, he had not had any serious illness. Three weeks before admission, while playing, he had struck his left groin with the edge of a chair. The immediate pain was slight and transient, and he continued work as a check-room boy. Two days after the injury, he began to have frequency and urgency of urination, and a lump was discovered in the left groin on the following day. He continued work, but five days after the injury, the lump became painful, and it was found that the temperature was elevated. Little could be found out about the patient's condition during the eleven days previous to admission to the hospital, except that deep respirations had caused pain in the region of the umbilicus; that he had contracted a slight cough and that he had had fever and, for the last four or five nights, night sweats. The pain in the groin had disappeared.

Physical Examination.—On admission, the patient had a hacking cough with little sputum, vague pain in the epigastrium, and generalized glandular enlargement, including the cervical, axillary, epitrochlear and inguinal glands. No single gland felt larger than 2 cm. in diameter. The largest mass in the left groin formed a nodular group from 3 to 4 cm. in diameter.

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* From the Pathological Laboratories of the Montreal General Hospital and the Department of Pathology, Harvard Medical School.

The tonsils were not visibly enlarged; the throat was normal. There was dulness to percussion on the right side in the axilla, and from the scapula to the base; impaired resonance was also noted at the base of the left lung. A friction sound was heard at the bases of both lungs, and many crepitant râles on both sides, low, and in the axilla on the right. There was some retrosternal dulness. The heart was not enlarged, and murmurs were not heard. The spleen was readily felt; it had a thin, sharp edge. A few red spots were present on the thorax, which were palpable; these did not resemble rose spots. The pulse rate was 100 and was dicrotic and regular. The temperature was 102 F. The blood count revealed: red cells, a normal number; white cells, 17,400 with 80 per cent polymorphonuclears. The urine was normal for albumin, sugar and casts; the specific gravity was 1.012. The Wassermann reaction was +++. Examination of the sputum, which was small in amount, showed it to be blood tinged; tubercle bacilli were not present; a few diplococci were found. A mouse that was given intraperitoneal injections remained well; it was killed and no exudate was found. Culture of the blood did not show growth in four days. The x-ray diagnosis (Dr. W. L. Ritchie) was as follows: "There are shadows throughout the lungs, particularly the bases, suggestive of infiltration due to tuberculosis. No evidence of any fluid. No mediastinal thickening. Heart normal in size, shape and position." The clinical impressions were: acute pneumonic phthisis with tuberculous adenitis, infectious mononucleosis or a lymphatic tumor. The Pirquet reaction was negative. A second blood count showed: red blood cells, 5,150,000; white blood cells, 19,800; polymorphonuclears, 88 per cent; lymphocytes, 11 per cent; large mononuclears, 1 per cent; platelets, 240,000, and reticulated red cells, 0.5 per cent. Two other counts gave similar results.

There were few other symptoms. The temperature ranged from 99.6 to 104 F., the pulse rate from 100 to 140, and the respiratory rate from 20 to 40; most of the time it was about 28. A second Pirquet reaction was negative. The dulness in the lung spread so that latterly the signs were those of a bilateral lobar pneumonia. The temperature dropped, the pulse rate mounted, and the patient died eleven days after admission, after an illness lasting thirty days.

Autopsy.—Autopsy was performed six hours after death. The body was that of a slender boy, whose stated age was 16, but who looked two years younger. It was well developed and well nourished, but there was no pubic hair, and little hair over the chest and axillae. The body measured 153 cm. in length. There was no rigor mortis or edema, and only slight lividity of the dependent parts. There was an abraded, reddened area about 4 cm. in diameter over the point of the right elbow. The scalp, eyes, ears, nose and mouth were normal. The pupils were equal and measured 7 mm. A comparatively large lobulated mass of nodes could be palpated in the inguinal regions of both sides. Smaller palpable nodes were present in each axilla and in the neck. The thyroid was not enlarged. The genital organs were small. The skin was smooth, pale and elastic. The subcutaneous fat measured about 1 cm. and was of normal color.

The peritoneum was moist throughout, and about 300 cc. of pale yellow, clear liquid was present in the pelvis. There were no adhesions, and the peritoneum was smooth. (Smears from the liquid contained a few large mononuclear cells, and bacteria were not revealed by Gram or acid-fast stains.) The abdominal organs were in their normal positions. The appendix did not show any lesion. The mesenteric lymph nodes were greatly enlarged and seemed to be increased in number, but were of about normal consistency (fig. 1). A cross-section showed only hyperplasia without evidence of tuberculosis.

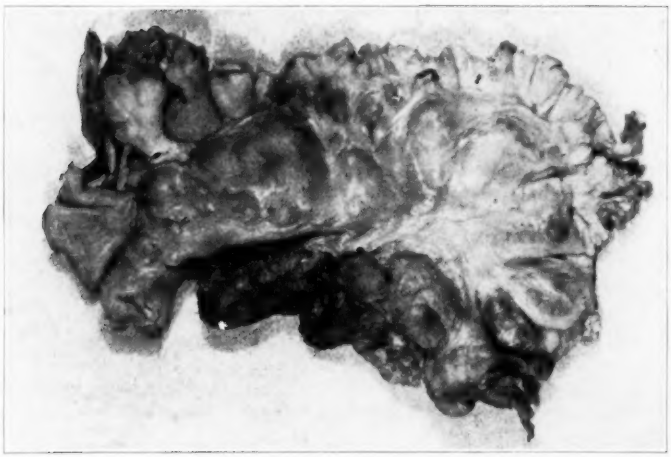

Fig. 1.—A mass of enlarged mesenteric lymph nodes; photograph after fixation.

Fig. 2.—Cut surface of the left lung showing generalized fibrosis. The right lung is similar in appearance; photograph after fixation by Kaiserling's method.

The pleural cavity contained about 30 cc. of liquid on each side, and in it there were a few flocculent masses. Both pleural surfaces were covered with a thin layer of fibrin. There were no old adhesions. The pericardial cavity contained clear liquid, which was not increased in amount. The heart weighed 240 Gm. The pericardium, endocardium, myocardium and valves did not show any gross lesions. The measurements were within normal limits.

The weight of the lungs was: right, 740 Gm., left, 600 Gm. Both lungs seemed to be completely consolidated. There was no place where crepitation could be made out. They were doughy and inelastic on palpation. The bronchi contained small amounts of mucoid exudate. A thin layer of fibrin covered most of the surfaces of both lungs. On section, the cut surface had a brick red, fleshy appear-

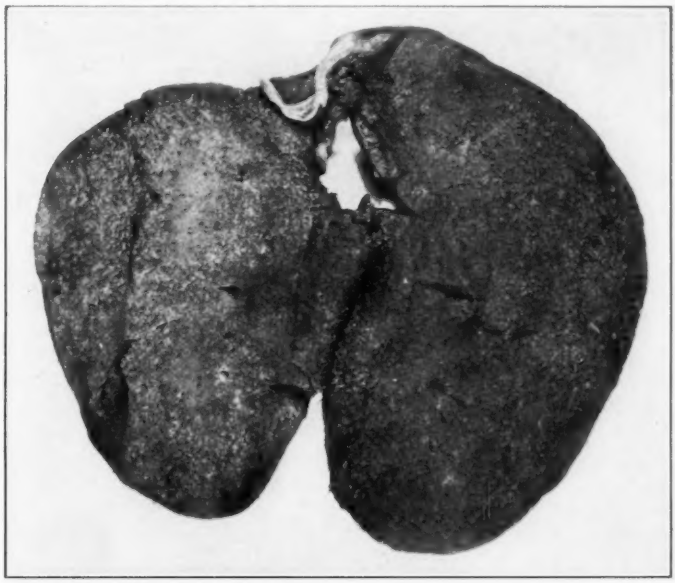

Fig. 3.—Photograph of spleen after Kaiserling fixation.

ance, and was somewhat granular. The granules were pale, lighter in color than the background, but were not palpable; they did not seem to be tubercles. The cut surface of both lungs was uniform, having the same appearance throughout (fig. 2). Large lesions, exudate or areas of caseation or of old fibrosis were not present. The lung was fairly dry; only a small amount of blood could be squeezed out, and no other liquid or pus.

The spleen weighed 380 Gm. It was greatly enlarged and brick red; the consistency was somewhat decreased over the normal. The capsule was of the usual thickness, smooth and not tense. The cut surface had a coarsely granular appearance, was grayish red, and had somewhat the appearance of the cut surface of the lung (fig. 3). The granules were barely palpable, pale and firm, but not tuberculous. The pulp was not excessive; little could be scraped away with the knife.

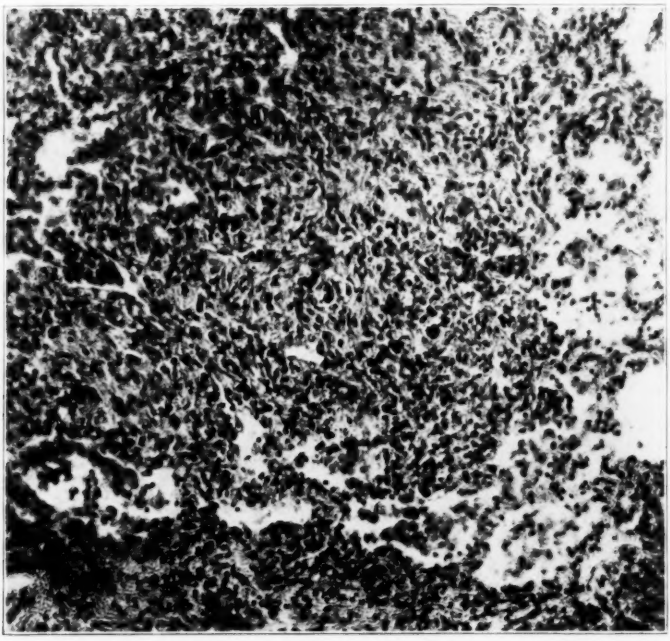

Fig. 4.—The appearance of the lung is that of a chronic progressing fibrosing condition rather than the organization of a previously acute inflammatory lesion. Giemsa stain; × 50.

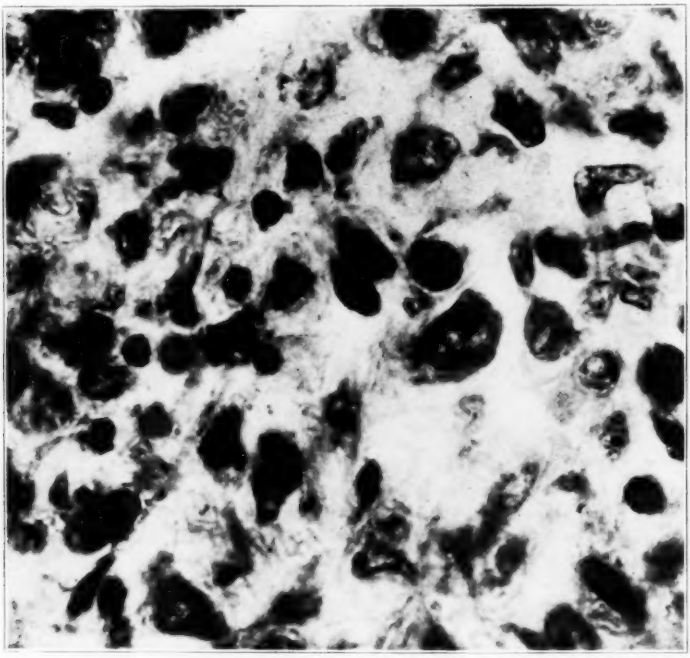

Fig. 5.—The types of cells present in the lung include endothelial cells forming capillaries, free endothelial leukocytes, fibroblasts and plasma cells. Giemsa stain; × 800.

The gastro-intestinal tract was opened from the esophagus to the anus but a lesion was not found. The pancreas itself was normal but around it, particularly along the splenic artery and at the head of the pancreas, there were numerous enlarged lymph nodes. The liver weighed 1,400 Gm. It seemed somewhat enlarged for the size of the child, but was normal in shape and in appearance. The cut surface was brownish gray, and showed the usual normal markings. The gall-bladder and ducts did not contain any lesions.

The weight of the kidneys was: right, 140 Gm., left, 150 Gm. They were essentially the same in appearance. The capsules stripped easily, showing a smooth surface. The cortex was 0.6 cm. in width, was regular and did not contain scars.

Fig. 6.—Fibroblastic proliferation in one of the splenic corpuscles. Hematoxylin and eosin stain; × 50.

The medulla and cortex showed moderate edema and congestion, but no other gross lesion.

There were no gross lesions in the suprarenals, bladder and genital organs. The testes were small and soft. The aorta was essentially normal. Around it the retroperitoneal glands were greatly enlarged up to the diaphragm. The bone marrow was red, although there were some small patches of fatty marrow. This was taken from the femur.

Several chains of lymph nodes were dissected out from both inguinal regions and from the neck. These were mostly soft and appeared to be hyperplastic without evidence of acute or chronic infection. One node from the inguinal region contained a mass of fibrous tissue in the center, and several others were rather firm and contained more fibrous tissue than usual. Caseation was not found in any

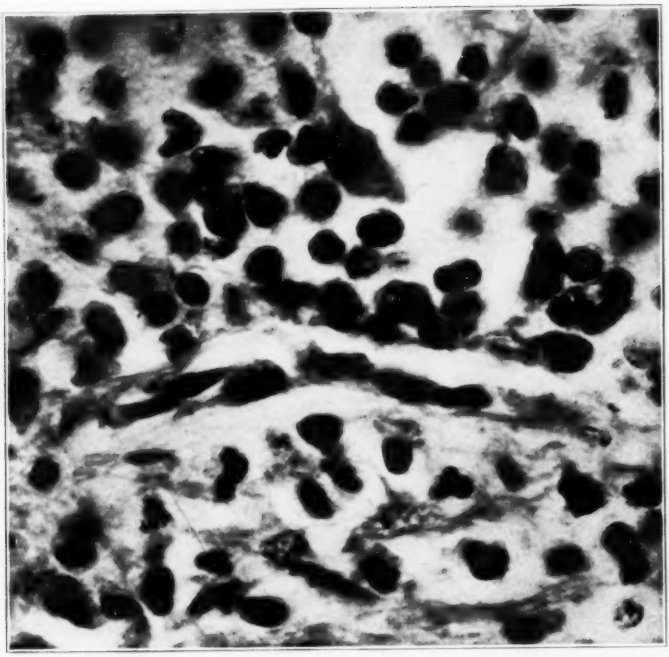

Fig. 7.—Endothelial proliferation and the character of the cells present in the spleen. Giemsa stain; × 800.

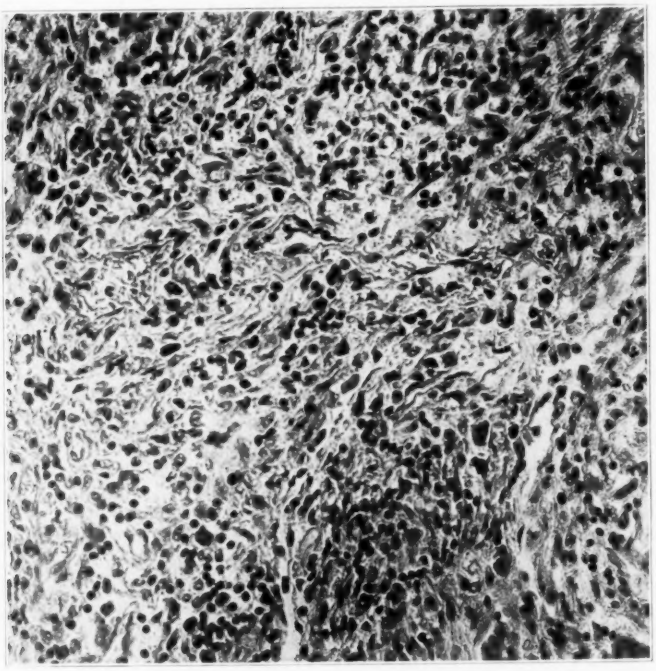

Fig. 8.—Proliferative lesion in the center of a lymphoid nodule. Giemsa stain; × 50.

of the nodes, nor was there any other evidence of a localized lesion within the nodes. This seemed to be a generalized lymphatic hyperplasia or tumefaction, the nature of which was not apparent from gross examination alone.

Microscopic Examination.—The pericardium and endocardium did not show any lesion. The muscle of the heart was well preserved. There was slight interstitial edema.

There was a peculiar organizing process throughout both lungs. Most of the alveolar walls were thickened with recent fibroblastic cells, and much of the lung substance had been replaced by fibrous tissue. The reaction was more interstitial

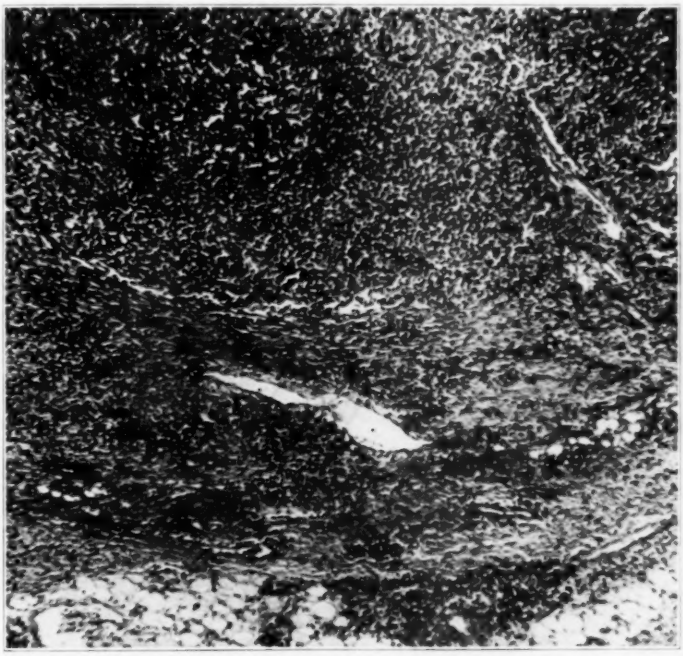

Fig. 9.—Mesenteric lymph node showing fibrosis of cortex, obstruction of peripheral sinuses, thickening and infiltration of the capsule and infiltration of the surrounding fat. Hematoxylin and eosin stain; × 20.

and peribronchial than alveolar. The few alveolar spaces remaining contained a large cell exudate or were clear. A few contained small amounts of hyaline fibrin. The picture was not that of the usual organizing pneumonia (fig. 4). The recently formed fibrous tissue did not follow the course of strands of fibrin in the usual manner. Many alveoli were plugged or obliterated by connective tissue and newly formed capillaries, but these plugs were continuous with and inseparable from the fibroblastic changes in the alveolar walls. Around the bronchi and bronchioles there were thick collars of fibrous tissue, and the alveoli adjacent to these seemed to be affected more than those at a distance.

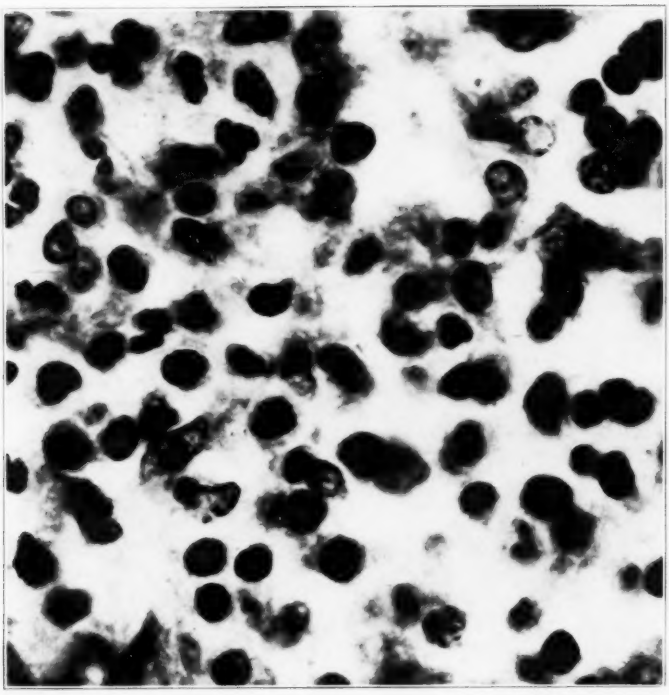

Fig. 10.—The character of the cells in the lymph nodes. Giemsa stain; × 800.

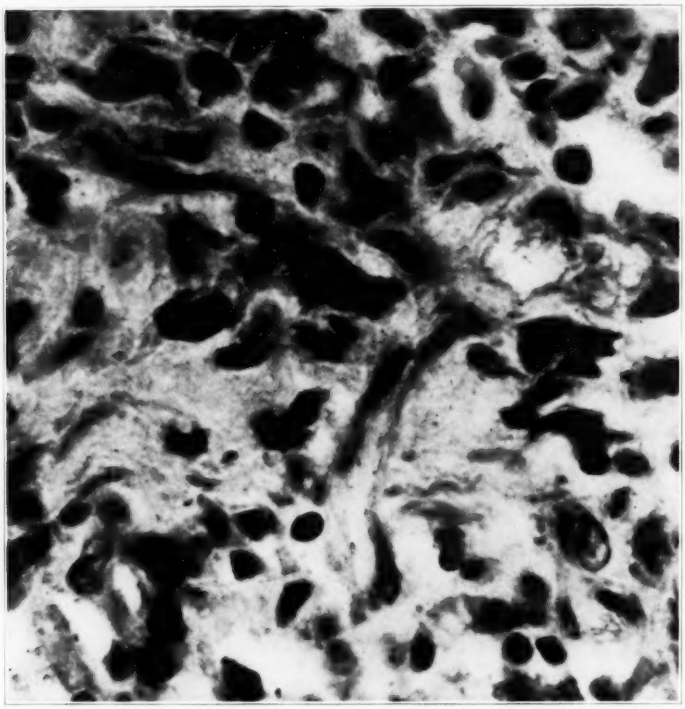

Fig. 11.—Endothelial and fibroblastic proliferation and capillary formation in lymph node. Phosphotungstic acid hematoxylin stain; × 800.

Embedded in, and intimately intermingled with, the fibroblasts were many endothelial cells. Some of these were forming capillaries, which were numerous; others were lying free in the tissue (fig. 5). A rare mitotic figure was seen among these cells. Other cells included numerous plasma cells and rare polymorphonuclear cells. Some of the bronchi contained small amounts of polymorphonuclear and mononuclear cell exudate.

Phosphotungstic acid hematoxylin and Giemsa's stain were used to study the intercellular substance and the cell types. These showed only more clearly the fibroblastic and endothelial cell proliferation, and emphasized the number of plasma cells present. Definite organisms could not be seen in the Giemsa prepara-

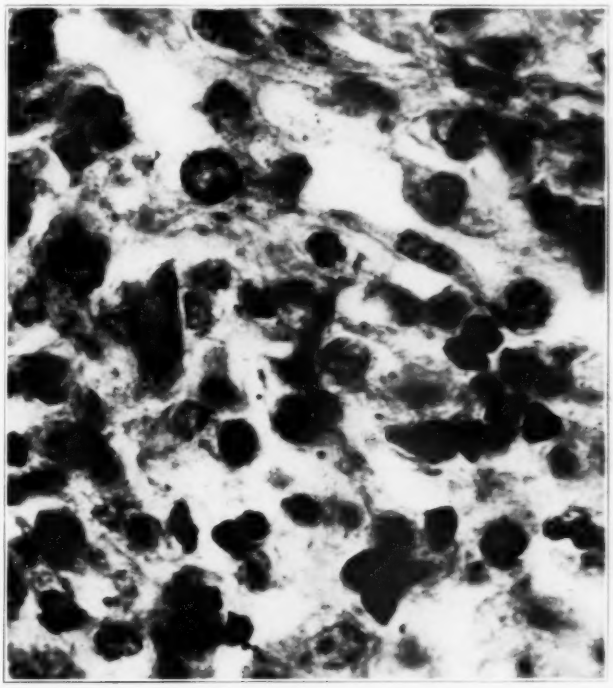

Fig. 12.—Cellular portion of lymph node resembling Hodgkin's disease, for comparison with figure 13. Giemsa stain; × 800.

tions, and bacilli were not found in sections stained for acid-fast organisms. Levaditi preparations did not show any spirochetes.

The pleura contained a thin layer of fibrin on the surface embedded in which were small numbers of polymorphonuclear cells and lymphocytes.

The spleen was cellular throughout, containing little blood. A fibrosing process was present which affected particularly the splenic corpuscles (fig. 6). These were present in increased numbers and prominence and most of them contained a proliferative lesion around the central arteriole. Here there was active fibroblastic and endothelial cell proliferation, intermingled with normal appearing

lymphocytes and a scattering of plasma cells and polymorphonuclear neutrophils (fig. 7). A few eosinophils were present, and infrequent, large, irregularly shaped cells with purple cytoplasm (hematoxylin and eosin) and large, irregularly shaped nuclei with reticulated chromatin (identical with Dorothy Reed cells) were also found. This same process was present in less degree throughout the spleen, obscuring the sinuses, although in general, the normal architecture was not greatly altered. The capillary endothelium was prominent, and rare mitotic figures were present in these cells. The splenic capsule was slightly or not at all thickened. Organisms could not be found in Giemsa and Levaditi preparations.

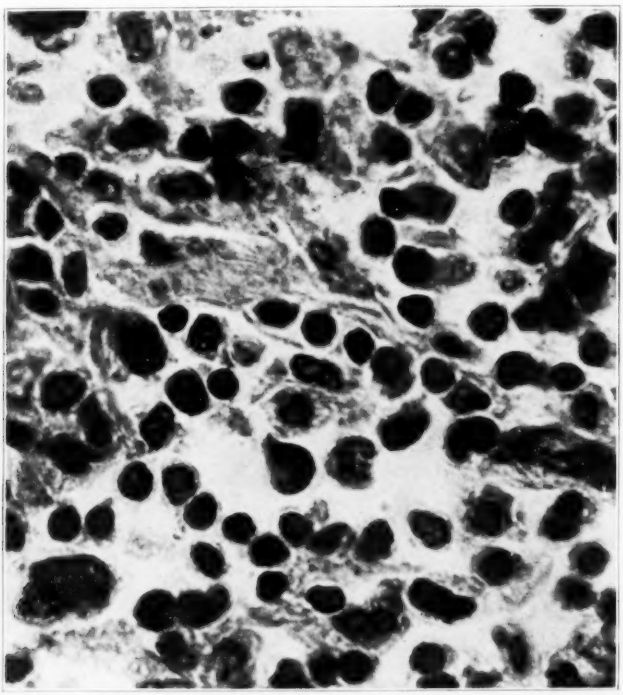

Fig. 13.—Section of cervical lymph node from a typical case of Hodgkin's disease, for comparison with figure 12. Hematoxylin and eosin stain; × 800.

The parenchyma of the liver appeared to be normal. There was no increase in the fibrous tissue, and no cellular infiltration. The bile ducts were normal. The pancreas and suprarenals did not show any lesions. The kidneys were only moderately congested. Recent or old lesions were not seen. There was no cellular infiltration. Spermatogenesis was not active. Spermatozoa were not present in the testis, and there was no proliferation of spermatids. The tubules were separated by wide bands of stroma, as were also the tubules of the epididymis. Insterstitial cells seemed to be present in the usual number. The bladder and prostate did not show any lesions.

Nearly all the nodes contained a granulomatous lesion in which there was more or less fibrosis (fig. 8). Few if any lymphoid nodules were present, and germinal centers were rare. In most of the glands the peripheral sinuses could not be seen; they were either collapsed or filled with fibrous tissue and cells. Many of the medullary sinuses were also obscured by an overgrowth of a similar nature, though some were recognizable. The capsules, as a rule, delimited the gland sharply, but one section showed two nodes with united capsules due to cellular and fibroblastic infiltration. A large mesenteric node showed cellular infiltration, not only into the thickened capsule, but also into the surrounding fat (fig. 9). The nodes showed fibroblastic-endothelial cell proliferation throughout, but in places

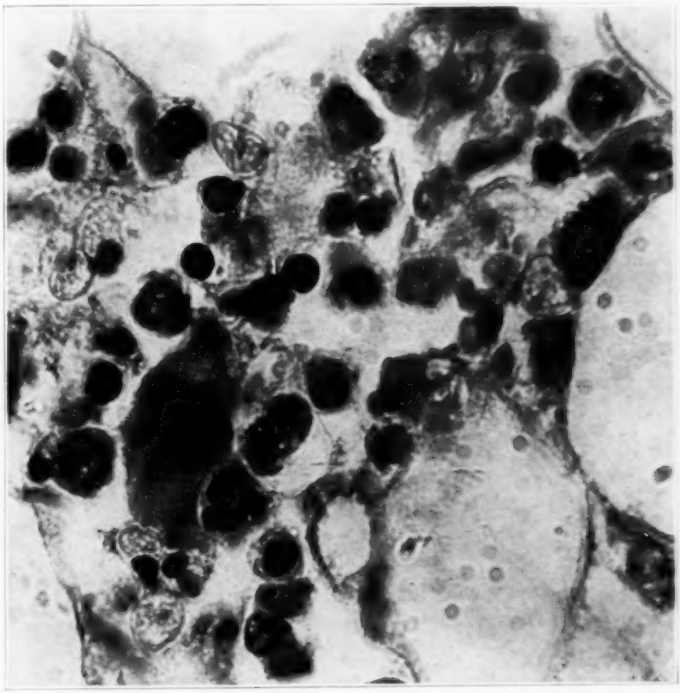

Fig. 14.—Endothelial (or reticular) hyperplasia, showing the predominant cell types in the bone marrow. Giemsa stain; × 800.

there was condensation of the connective tissue, mostly in what appeared to be the centers of obliterated lymphoid nodules. The cells present included endothelial cells, plasma cells, lymphocytes and rare polymorphonuclear neutrophils (fig. 10). Eosinophils were extremely rare; only one or two were found in eight sections. Large, irregularly shaped cells with large nuclei, occasionally two nuclei, were infrequent, but a few were seen in each node. These were identical with the tumor cells (Dorothy Reed cells) of Hodgkin's disease. Mitotic figures were not numerous, but were seen in endothelial cells and in other undifferentiated cells

(figs. 11, 12, 13). The exact nature of the lesion could not be determined. It resembled Hodgkin's disease, but in general was more of a chronic inflammatory lesion.

There was moderate diffuse hyperplasia of the bone marrow. Most of the fat cells were separated by distended sinusoids filled with cells. These cells were mostly of one type—fairly large cells with purple cytoplasm and round nuclei (fig. 14). In most cells the chromatin was abundant, but scattered, forming no particular pattern within the nucleus. Cells of similar appearance were seen attached to the walls of capillaries and sinuses. Myelocytes were rare, and no nucleated red cells were seen. Megakaryocytes were present in normal or in slightly increased numbers. Pigment was not present, and organisms were not seen in Giemsa preparations.

SUMMARY

The case of a boy with a chronic progressive condition of the lungs, lymph nodes and spleen which lasted for thirty days, and which ended with massive consolidation of the lungs is recorded. At autopsy there was widespread enlargement of the lymph node, a diffuse fibrosis of both lungs and a large somewhat granular spleen. The bone marrow was moderately hyperplastic. Histologically, there were fibrosis, endothelial cell proliferation and cellular infiltration of the lymph nodes of a definitely inflammatory character, but in some places closely resembling the sclerosing lymphosarcoma of Hodgkins' disease. The spleen and lungs showed similar pictures, the histology of the lung being that of a chronic progressing fibrosis rather than the organization of a previous acute exudative lesion. The causative organism was not found.

EXPERIMENTAL UREMIA-UREMIC ENTERITIS*

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The purpose of this experimental study was (*a*) to produce a disturbance in a dog that parallels the clinical features of uremia; (*b*) to study the effects of such a disturbance on the gastro-intestinal canal, and (*c*) to arrive at a possible explanation of uremia on a chemical basis. As urea is the principal constituent of the urine, I naturally employed this metabolite as the uremia-producing substance.

Visceral disturbances in uremia are frequently so pronounced that the writers of the nineteenth century often referred to this condition as visceral uremia. The literature on this subject is profuse and at wide variance. I, therefore, will consider only a few references which deal directly with the problem.

REVIEW OF THE LITERATURE

The chemical theory of Wilson in 1833 ascribes uremia as due to the retention of urea in the blood stream. This theory met with considerable opposition and was primarily contested by Frerichs (1852), Herker (1898), Ritter (1880) and Bouchard (1887).

In 1860, the anatomic theory became temporarily popular through the researches of Traube, who believed that uremia was caused by edema of the brain and consequent disturbances in circulation.

In 1889, Brown-Sequard published his theory on internal secretion and described the causation of uremia as due to failure of kidney secretion.

Since 1889, little advance had been made on the subject until 1912, when Folin and his co-workers[1] introduced accurate methods for chemical analysis of the blood and the urine.

Considerable data have been accumulated by Marshall,[2] Van Slyke,[3] Myers and Fine,[4] Denis, Austin and Farr,[5] McLean and Selling,[6]

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1. Folin and Farmer: J. Biol. Chem. **11**:493, 1912. Folin: ibid. **11**:507, 1912. Folin and Macallum: ibid. **11**:523, 1912. Folin and Denis: ibid. **11**:527, 1912.

2. Marshall: J. Biol. Chem. **14**:283, 1913; ibid. **15**:487, 1913; ibid. **15**:495, 1913.

3. Van Slyke and Cullen: J. Biol. Chem. **19**:211, 1914; ib'd. **24**:117, 1916.

4. Myers and Fine: J. Biol. Chem. **20**:391, 1915.

5. Farr and Austin: J. Exper. Med. **18**:228, 1913.

6. McLean and Selling: J. Biol. Chem. **19**:31, 1914.

Schwartz and McGill [7] and Chace and Myers [8] on the retention of nonprotein nitrogen, urea nitrogen and uric acid. It is interesting to note that Vanquelin and Segales, in 1822, were the first to record intravenous injections of urea in animals. In 1858, Hammond interfered with the kidneys while making injections of urea, and found that the injections were fatal. In some animals, one ureter was ligated and it was found that after a short time the remaining functioning kidney would take care of as much urea as both kidneys previous to the operation, indicating a high margin of safety. In 1920, Wells [9] was inclined to the view that uremia was caused by the retention of the known nitrogenous substances in the blood, and emphasized especially the importance of the time element.

Experiments with the Woodyatt machine to determine the effects of prolonged intravenous injections of the various purified urinary constituents were recommended as being highly essential for further progress. An interesting set of experiments was carried out by Andrews [10] (1927), in which a chemical picture simulating uremia was reproduced by intravenous injection of a 5 per cent saline solution.

MATERIALS AND METHODS

Dogs were repeatedly injected with from 10 to 20 per cent urea (Merck) solution (at body temperature) intravenously; 200 cc. was given daily for three days at the rate of from 1 to 5 cc. a minute. Dietary restrictions were not used except in one group of controls.

General anesthesia was necessary only in some instances. The injections were made by means of a Woodyatt pump into the femoral vein. No evidence of hemolysis was present. The animals were kept in individual cages and were carefully controlled; samples of the heart's blood, feces and urine of each animal were collected at twenty-four hour intervals for chemical analysis. Necropsy was performed on all the dogs soon after the death. The viscera were examined and sections were made for histologic study.

One ureter had been ligated in many instances.

EXPERIMENTAL WORK

In about 40 per cent of the dogs, a picture similar to that of uremic coma, the so-called "Kussmaul picture," was produced after a single injection of 200 cc. of 10 or 20 per cent solution of urea, and in practically 100 per cent of the animals, coma was invariably produced after the second or third injection.

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7. Schwartz and McGill: Blood Urea, Arch. Int. Med. **17**:42 (Jan.) 1916.

8. Chace, A. F., and Myers, V. C.: The Value of Recent Laboratory Tests in the Diagnosis and Treatment of Nephritis, J. A. M. A. **67**:929 (Sept. 23) 1916.

9. Wells: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1920.

10. Andrews, Edmund: Experimental Uremia, Arch. Int. Med. **40**:548 (Oct.) 1927.

A representative example of each group of experiments is included in the following reports.

The experiments on dog 1 represent a group in which the right ureter had been ligated and food has been allowed.

Dog 1.—A female dog, weighing 15.6 Kg. received 200 cc. of 10 per cent urea solution daily for three days. Salivation was noticed after 100 cc. of the solution was injected. Vomiting occurred after 200 cc. was injected, and dyspnea and restlessness after the injection of 300 cc.

An injection of 400 cc. of the solution produced generalized tremors and 500 cc., convulsions.

Bloody diarrhea, retraction of the head and neck, unconsciousness and death followed the injection of 600 cc. of the solution of urea.

The chemical analysis of this animal is included in the group represented by table 1.

The necropsy report revealed that the entire gastro-intestinal canal was markedly injected and hyperemic; there was no evidence of ulcerations. The left kidney was apparently normal in gross. The right kidney was distended with fluid and the medullary substance disappeared entirely. The lungs showed marked hyperemia. The liver was dark and full of blood.

Histologic examination showed marked enteritis of the gastro-intestinal canal. The liver was congested, and there was marked engorgement. Cloudy swelling of epithelium, and hyperemia were noted in the kidney. Hyperemia was observed in the lung. No other changes were noted.

Dog 2 is representative of a group in which the right ureter had been ligated, but no food given.

Dog 2.—A female dog, weighing 14.3 Kg., received 200 cc. of a 10 per cent solution of urea daily for three days. The animal followed the same course as dog 1. The chemical analysis is demonstrated by table 2. The necropsy observations and results of the histologic examination were identical with those made on dog 1.

This set of experiments was instituted to determine what effect, if any, limitation of food would have on the chemical analysis of the excretory products.

Dog 3 is representative of a group in which the right ureter had been ligated and food was given, and dog 4 of the group in which both ureters remained intact, and food given.

Dog 3.—A female, weighing 16.5 Kg., received 200 cc. of a 20 per cent solution daily for three days (table 3).

Dog 4.—A female, weighing 16.2 Kg., received 200 cc. of a 20 per cent solution daily for three days (table 4).

RESULTS

The chemical observations made during the various sets of experiments are shown in figures 1 and 2.

TABLE 1.—*Chemical Analysis of Intravenous Injection of Urea Solution as in Dog 1**

	Before Injection			24 Hours After Injection			48 Hours After Injection			72 Hours After Injection			
	Urine	Blood	Feces	Urine	Blood	Feces	Urine	Blood	Feces	Urine	Blood	Feces	Remarks
Output	600 cc.	.....	24 Gm.	200 cc.	.....	54 Gm.	1,000 cc.	.....	7 Gm.	1,250 cc.	.....	18 Gm.	Right ureter ligated; average weight of dog 16 Kg.; (female, food given); series of 6 dogs; convulsions; Cheyne-Stokes breathing; diarrhea; coma; death
Total nitrogen	3.2 Gm.	.....	5.1 Gm.	4.9 Gm.	.....	2.4 Gm.	13.0 Gm.	.....	0.3 Gm.	9.5 Gm.	.....	4.1 Gm.	
Ammonia nitrogen	0.46 Gm.	.....	0.01 Gm.	0.19 Gm.	.....	0.13 Gm.	1.9 Gm.	.....	0.02 Gm.	2.4 Gm.	.....	0.09 Gm.	
Urea nitrogen	0.4 Gm.	24 mg.	0.03 Gm.	3.2 Gm.	38 mg.	0.0 Gm.	10.2 Gm.	35 mg.	0.1 Gm.	6.4 Gm.	22 mg.	0.02 Gm.	
Albumin	—	..	..	+	..	..	+	..	..	+	..	..	
Carbon dioxide	.....	51.2 cc.	.....	.....	40.1 cc.	.....	.....	36.3 cc.	.....	.....	30.4 cc.	.....	
Chlorides	.....	389 mg.	.....	.....	425 mg.	.....	.....	501 mg.	.....	.....	528 mg.	.....	
Calcium	.....	6.3 mg.	.....	.....	17.4 mg.	.....	.....	10.1 mg.	.....	.....	8.2 mg.	.....	
Potassium	.....	25.1 mg.	.....	.....	23 mg.	.....	.....	19.0 mg.	.....	.....	21.0 mg.	.....	
Index of refraction	.....	58	.....	.....	54	.....	.....	51	.....	.....	51	.....	

* Two hundred cubic centimeters of 10 per cent solution was given daily for three days; rate of injection, 5 cc. a minute.

TABLE 2.—*Chemical Analysis of Intravenous Injection of Urea Solution as in Dog 2**

	Before Injection			24 Hours After Injection			48 Hours After Injection			72 Hours After Injection			
	Urine	Blood	Feces	Urine	Blood	Feces	Urine	Blood	Feces	Urine	Blood	Feces	Remarks
Output	700 cc.	.....	28 Gm.	500 cc.	.....	32 Gm.	500 cc.	.....	18.3 Gm.	600 cc.	.....	14 Gm.	Right ureter ligated; average weight of dog 15 Kg.; (female, no food given); series of 6 dogs; convulsions; vomiting; Cheyne-Stokes breathing; diarrhea; coma; death
Total nitrogen	3.4 Gm.	.....	6.0 Gm.	7.12 Gm.	.....	8.1 Gm.	4.6 Gm.	.....	3.2 Gm.	6.1 Gm.	.....	2.3 Gm.	
Ammonia nitrogen	0.58 Gm.	.....	0.05 Gm.	0.14 Gm.	.....	0.14 Gm.	0.21 Gm.	.....	0.109 Gm.	1.2 Gm.	.....	0.07 Gm.	
Urea nitrogen	0.48 Gm.	18 mg.	0.04 Gm.	4.8 Gm.	96 mg.	0.0 Gm.	3.6 Gm.	45 mg.	0 Gm.	3.1 Gm.	21 mg.	0.03 Gm.	
Albumin	—	..	..	+	..	..	+	..	..	+	..	..	
Carbon dioxide	.....	45.7 cc.	.....	.....	44.7 cc.	.....	.....	30.5 cc.	.....	.....	30.4 cc.	.....	
Chlorides	.....	440 mg.	.....	.....	501 mg.	.....	.....	711 mg.	.....	.....	680 mg.	.....	
Calcium	.....	7.2 mg.	.....	.....	21.3 mg.	.....	.....	19.1 mg.	.....	.....	9.6 mg.	.....	
Potassium	.....	21.9 mg.	.....	.....	17.4 mg.	.....	.....	16.4 mg.	.....	.....	18.4 mg.	.....	
Index of refraction	.....	58	.....	.....	54	.....	.....	50	.....	.....	51	.....	

* Two hundred cubic centimeters of 10 per cent solution was given daily for three days; rate of injection, 5 cc. a minute.

TABLE 3.—*Chemical Analysis of Intravenous Injection of Urea Solution as in Dog 3**

	Before Injection			24 Hours After Injection			48 Hours After Injection			72 Hours After Injection			
	Urine	Blood	Feces	Urine	Blood	Feces	Urine	Blood	Feces	Urine	Blood	Feces	Remarks
Output	700 cc.	.....	31 Gm.	260 cc.	.....	69 Gm.	480 cc.	.....	80 Gm.	650 cc.	.....	68 Gm.	Right ureter ligated; average weight of dog 17 Kg.; female, food given; series of 15 dogs; convulsions; vomiting; diarrhea; increased respiration; coma; death
Total nitrogen	4.7 Gm.	.....	3.9 Gm.	5.9 Gm.	.....	2.1 Gm.	13.9 Gm.	.....	1.8 Gm.	11.9 Gm.	.....	1.4 Gm.	
Ammonia nitrogen	0.4 Gm.	.....	0.02 Gm.	0.5 Gm.	.....	0.13 Gm.	0.38 Gm.	.....	0.35 Gm.	1.6 Gm.	.....	0.34 Gm.	
Urea nitrogen	0.38 Gm.	19 mg.	0.04 Gm.	4.5 Gm.	42 mg.	0.06 Gm.	10.4 Gm.	88 mg.	0.1 Gm.	7.1 Gm.	76 mg.	0.08 Gm.	
Albumin	—	..	..	+	..	..	+	.....	.....	+	.....	.....	
Carbon dioxide	.....	52.4 cc.	.....	.....	46.1 cc.	.....	.....	40.8 cc.	.....	.....	52.1 cc.	.....	
Chlorides	.....	462 mg.	.....	.....	552 mg.	.....	.....	533 mg.	.....	.....	492 mg.	.....	
Calcium	.....	8.2 mg.	.....	.....	19.3 mg.	.....	.....	24.4 mg.	.....	.....	11.3 mg.	.....	
Potassium	.....	20.2 mg.	.....	.....	14.7 mg.	.....	.....	13.1 mg.	.....	.....	23.4 mg.	.....	
Index of refraction	.....	50	.....	.....	51	.....	.....	45	.....	.....	50	.....	

* Two hundred cubic centimeters of 20 per cent solution was given daily for three days; rate of injection, 5 cc. a minute.

TABLE 4.—*Experimental Uremia: Chemical Analysis of Intravenous Injection of Urea Solution as in Dog 4**

	Before Injection			24 Hours After Injection			48 Hours After Injection			72 Hours After Injection			
	Urine	Blood	Feces	Urine	Blood	Feces	Urine	Blood	Feces	Urine	Blood	Feces	Remarks
Output	750 cc.	.....	22 Gm.	600 cc.	.....	11 Gm.	890 cc.	.....	16 Gm.	750 cc.	.....	9 Gm.	Ureter not ligated; average weight of dog 17 Kg.; female; food given; series of 9 dogs; convulsions; vomiting; Cheyne-Stokes breathing; diarrhea; coma; death
Total nitrogen	4.1 Gm.	.....	4.0 Gm.	6.8 Gm.	.....	1.8 Gm.	22.8 Gm.	.....	2.3 Gm.	16.5 Gm.	.....	1.1 Gm.	
Ammonia nitrogen	0.3 Gm.	.....	0.03 Gm.	0.6 Gm.	.....	0.1 Gm.	0.48 Gm.	.....	0.2 Gm.	0.6 Gm.	.....	0.1 Gm.	
Urea nitrogen	0.4 Gm.	18 mg.	0.05 Gm.	4.4 Gm.	49 mg.	0.02 Gm.	21.0 Gm.	129 mg.	0.1 Gm.	12 Gm.	148 mg.	0 Gm.	
Albumin	—	..	..	+	..	..	+	.....	.....	+	.....	.....	
Carbon dioxide	.....	50.8 cc.	.....	.....	41 cc.	.....	.....	37 cc.	.....	.....	32 cc.	.....	
Chlorides	.....	418 mg.	.....	.....	531 mg.	.....	.....	504 mg.	.....	.....	673 mg.	.....	
Calcium	.....	8.1 mg.	.....	.....	15.4 mg.	.....	.....	18.50 mg.	.....	.....	16.2 mg.	.....	
Potassium	.....	20 mg.	.....	.....	17.4 mg.	.....	.....	14 mg.	.....	.....	13.1 mg.	.....	
Index of refraction	.....	54	.....	.....	51	.....	.....	51	.....	.....	48	.....	

* Two hundred cubic centimeters of 20 per cent solution was given daily for three days; rate of injection, 5 cc. a minute.

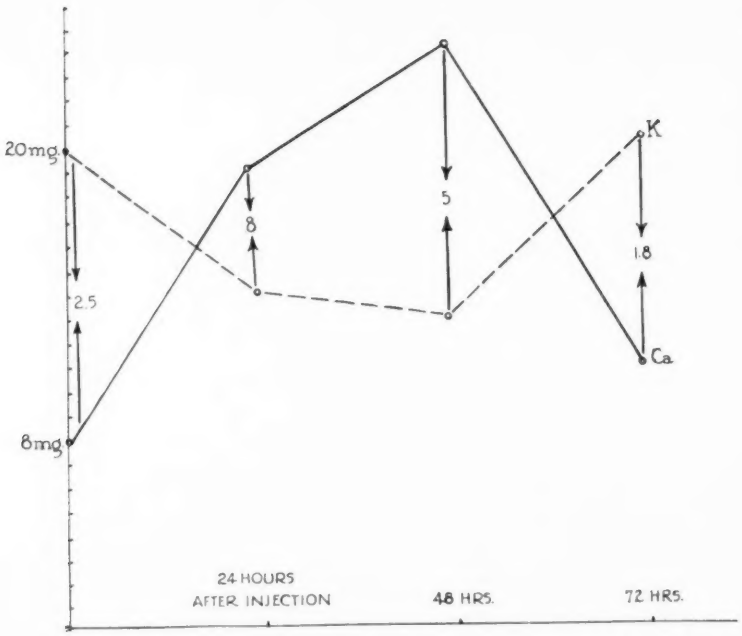

Fig. 1.—Experimental uremia, showing the calcium-postassium ratio. In this case the right ureter was ligated.

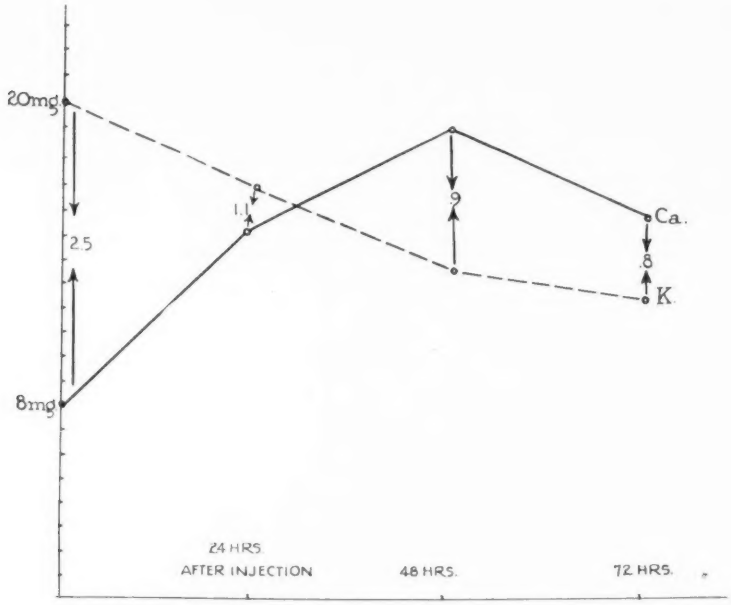

Fig. 2.—Experimental uremia, showing the calcium-potassium ratio. In this case the ureter was not ligated.

The outstanding features of these experiments are as follows: (*a*) a progressive acidosis demonstrated by a marked fall in carbon dioxide content; (*b*) a marked fall in proteins in the blood is evident in the decrease of the refractometric index; (*c*) marked rise in urine chlorides; (*d*) disturbance in the calcium—potassium ratio (the ratio approaching 1 or 1 plus); (*e*) marked increase in urea in the blood; (*f*) a positive (++) test for albumin in urine is constant.

Table 4 gives the chemical analysis of nine dogs in which the ureter had not been ligated. It is of interest to point out the marked parallelism of these results as compared with results in all other animals in which the ureter had been ligated.

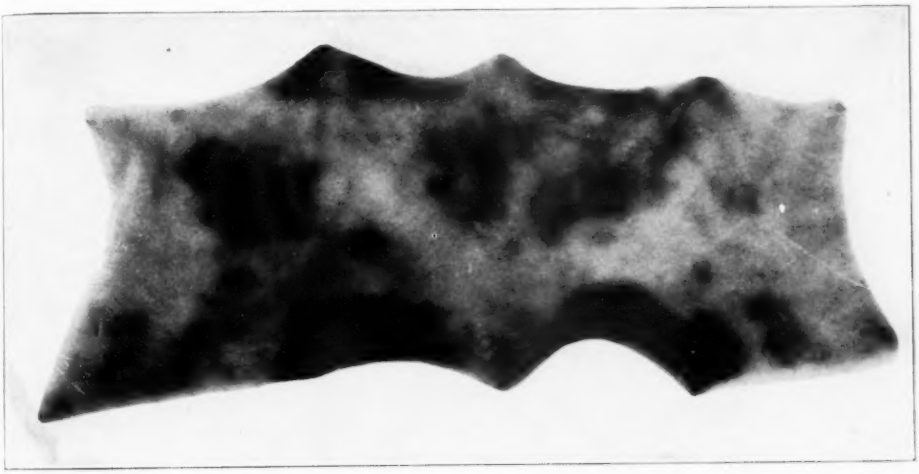

Fig. 3.—Pathologic changes in the intestine—a marked enteritis and congestion. There is no evidence of ulceration.

The pathologic observations made from the experiments are shown in figures 3, 4, 5 and 6.

No other observations of consequence were found and, therefore, other examinations of the tissues are not reported.

COMMENT

Urea.—On careful analysis of the output of urea in the form of ammonia nitrogen and urea nitrogen, it is evident that only about 25 per cent is recovered in the urine and feces; therefore, the concentration of urea in the blood was studied carefully. Two hundred cubic centimeters of a 20 per cent solution of urea was injected intravenously

into a dog (female) for three days and frequent chemical determinations were made for urea in the heart's blood (fig. 7).

It is clearly demonstrated that the urea in the blood is markedly elevated after a single injection (175 mg. in thirty minutes), and that the peak reaches 400 mg. after the second injection and 700 mg. after the third injection.

Calcium-Potassium Ratio.—The most striking features in the chemical studies were the profound changes in the mineral salt balance,

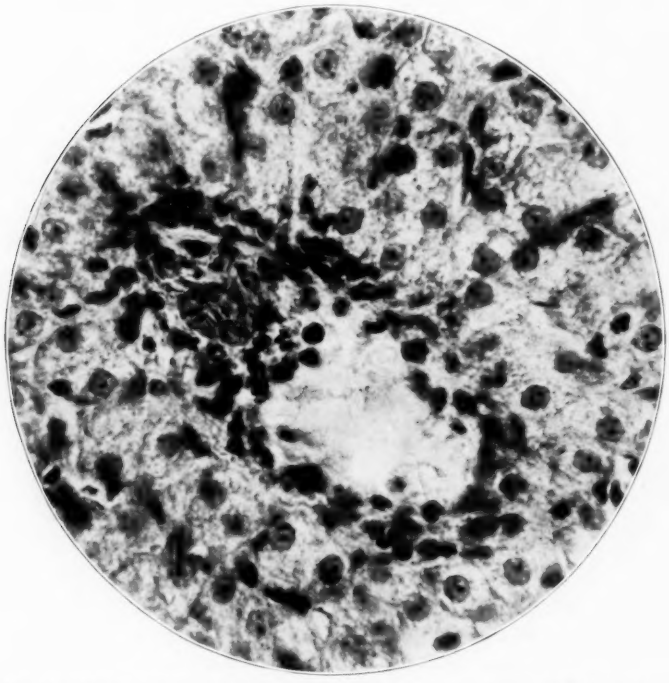

Fig. 4.—Marked engorgement and congestion of the liver. Cellular changes are not observed.

brought about by the injections of urea. An almost unprecedented rise in the calcium of the blood takes place, accompanied by a correspondingly profound fall in the potassium. So marked are these changes that the potassium-calcium ratio falls from a normal about 2.5 to levels far below 1 (figs. 1 and 2).

It is striking that these changes in permeability correspond exactly with those reported in Andrews' "Experimental Uremia" which were produced by solutions of sodium chloride. One is lead to believe that the

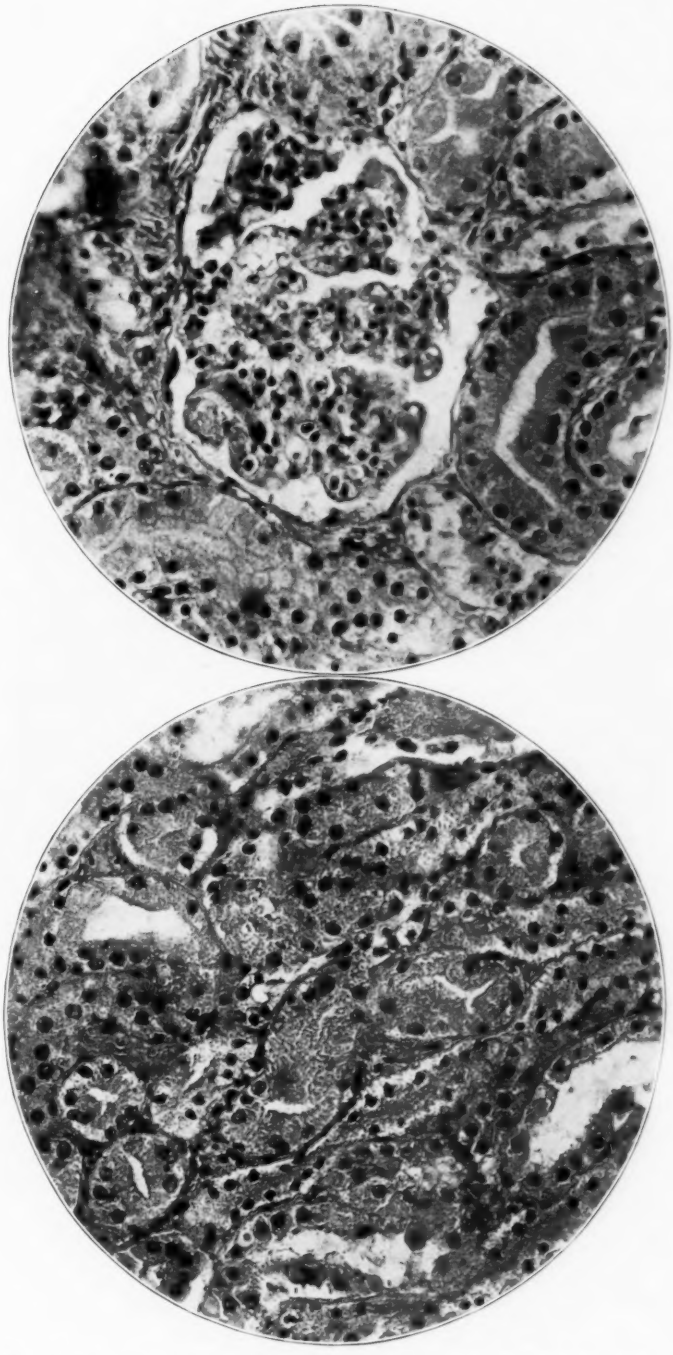

Fig. 5.—Photomicrographs of a kidney showing normal glomerulus and several convoluted tubules in which marked obstruction of the lumen is evident. Considerable hyperemia and marked cloudy swelling of the epithelium may be noted.

injections of urea bring about disturbances of the osmotic equilibrium under conditions of acidosis much the same as the sodium chloride will, and that the mechanism of production of the uremic symptoms is the same; that is, that owing to changes in cell permeability, toxic nitrogenous products are allowed to leak into the circulation.

Although slight histologic changes in the liver were noted in my experiments, the urine from two of the animals was studied immunologically by Dr. Thomas and shown to react in high dilutions to liver protein.

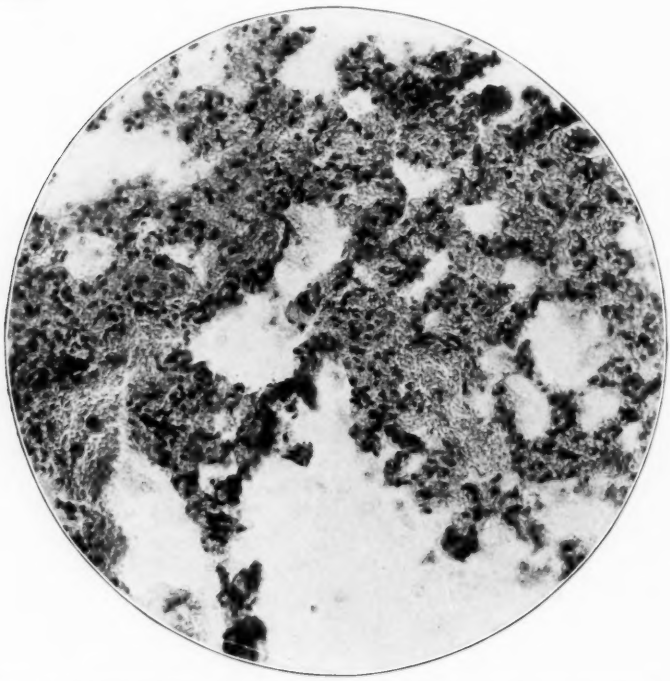

Fig. 6.—Showing considerable edema of the alveolar walls and hyperemia of the lungs.

Carbon Dioxide Content.—The decrease in carbon dioxide from about 50 to 30 cc. was constant and strikingly similar to that of the acidosis of uremic coma in the human being.

Kussmaul Picture.—The usual progression of symptoms of muscle irritability, albuminuria, acidosis, vomiting, diarrhea, convulsions, Cheyne-Stokes breathing and coma and death, appeared constantly regardless of whether the ureter had been ligated or remained intact.

With this in mind and the fact that on histologic survey so few changes were found in the kidneys, I am inclined to believe that involvement of the kidney is not pertinent to the production in a dog of disturbance which markedly parallels uremic coma in the human being.

Blood Pressure.—The blood pressure invariably went up in the animals used in these experiments, rising as high as 200 or 220 mm. (McGregor method).

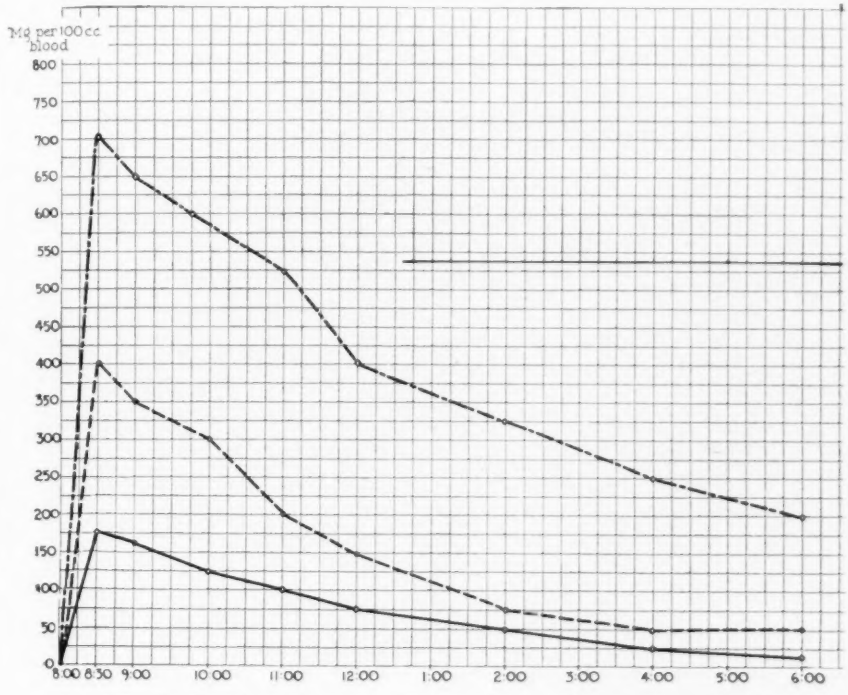

Fig. 7.—Experimental uremia. Showing the reaction of urea in the blood after the intravenous injection of a 20 per cent solution of urea three times daily.

CONCLUSIONS

1. Clinical uremia may be reproduced in all its features by the intravenous injection of from 10 to 20 per cent of a solution of urea.

2. Intravenous injections of from 10 to 20 per cent of a solution of urea produces a marked enteritis.

3. All features of uremia may be reproduced in presence of both kidneys.

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A REVIEW OF THE TECHNICAL METHODS OF DEMONSTRATING THE CIRCULATION OF THE HEART

A MODIFICATION OF THE CELLULOID AND CORROSION TECHNIC

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Many different methods have been employed in the anatomic study of the blood vessels, and all of these have been applied at one time or another in the study of the coronary circulation. The most important of these procedures may be grouped under the following headings:

1. Dissection.
2. Intravascular injection of dyes and other substances without clearing, roentgenography or corrosion.
3. Serial sections.
4. Impregnation methods.
5. Injection of an opaque substance followed by roentgenography.
6. Injection of a suitable mass followed by clearing.
7. Injection of a comparatively stable mass followed by maceration or corrosion.

REVIEW OF TECHNICAL METHODS

Dissection, in the hands of Galen, Vieussens and Haller, gave the first knowledge of the blood supply of the heart. This method, although its limitations are at once apparent, has been and always will be utilized by every earnest student of the coronary circulation. A systematic consideration of this field cannot be complete without careful control by dissection.

The injection of dyes and other substances for the purpose of recognizing the blood vessels, in gross as well as in microscopic sections,

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1. Vieussens, quoted by Dragneff (footnote 12, first reference).

2. Lee, A. B.: The Microtomist's Vade-Mecum, ed. 7, Philadelphia, P. Blakiston's Son & Company, 1913, p. 258.

3. Bellarminow: Zur Technik der Korrosion von Celloidinpräparaten, Anat. Anz. **3**:650, 1888. Hinman, Frank; Morison, D. M., and Lee-Brown, R. K.: Methods of Demonstrating the Circulation in General, J. A. M. A. **81**:177 (July 21) 1923. Polano, O.: Beiträge zur Anatomie der Lymphbahnen im menschlichen Eierstock, Monatschr. f. Geburtsh. u. Gynäk. **17**:281, 1903.

4. Mayer, Paul: Zoomikrotechnik, Berlin, Gebrüder Bornbraeger, 1920, p. 516. Schneider, quoted by Mayer. Terry, W. I., and Delamere, G. S.: Arterial Circulation of Thyroid Adenomas, Arch. Surg. **8**:165 (Jan.) 1924. Wearn, J. T.: Extent of Capillary Bed and Rôle of Thebesian Vessels in Coronary Circulation, Proc. Soc. Exper. Biol. & Med. **23**:707, 1925-1926.

has been extensively used for many years. As this is one of the older methods, various substances have been utilized, from time to time, to suit the particular purpose of the various investigators, some of whom employed dyes such as saffron,[1] carmine,[2] Prussian blue,[3] and India ink [4] and silver nitrate,[5] in aqueous solutions, in gelatin [6] or mixed with various other ingredients.[7] Others have experimented with injection masses including wax,[8] starch mixtures,[9] plaster of Paris,[10] glue,[11] glazier's putty [12] (Teichmann's linseed oil masses [13]), asphalt [14] (dissolved in benzene, ether or chloroform), India rubber [15] (in carbon bisulphide); gum arabic [16] (Bjeloussow's mass), sodium silicate,[17] oil of sesame,[18] shellac [19]

5. Golubew, W. Z.: Ueber die Blutgefässe der Niere der Säugetiere und des Menschen, Internat. Monatschr. f. Anat. u. Physiol. **10**:547, 1893.

6. Amenomiya, R.: Ueber die Beziehungen zwischen Koronararterien und Papillarmuskeln im Herzen, Virchow's Arch. f. path. Anat. **199**:187, 1910. Golubew (footnote 5). Müller, W.: Injectionsmassen von Thiersch und W. Müller, Arch. f. mikr. Anat. **1**:148, 1865. Rouhault, Simon, quoted by Unna (footnote 7, second reference).

7. Gerota: Zur Technik der Lymphgefässinjection: Eine neue Injectionmasse für Lymphgefässe-Polychrome Injection, Anat. Anz. **12**:216, 1896. Unna, Paul: Untersuchungen über die Lymph- und Blut-gefässe der äusseren Haut mit besonderer Berücksichtigung der Haarfollikel, Arch. f. mikr. Anat. **72**:161, 1908.

8. Abernethy, J.: Observations on the Foramina Thebesii of the Heart, Phil. Tr., London, 1798, p. 103. Munroe, A., quoted by Unna (footnote 7, second reference). Swammerdamm, quoted by Brühl (footnote 48) and Unna (footnote 7, second reference).

9. Gage, S. H.: Starch Injection-Mass, J. Roy. Micr. Soc. **8**:1056, 1888. Meyer, Hermann: Modificirte Form der Kleisterinjection, Arch. f. Anat. u. Entwcklngsgesch., 1882, p. 60; Fernere Mittheilung über die Kleisterinjection, ibid, 1883, p. 277. Pansch, A.: Noch einmal die Kleisterinjection, ibid. **6**:76, 1881; Kalte Injection mit Kleistermasse, ibid., 1877, p. 480. Wikszemski, Adam: Eine Modification der von Pansch empfohlenen kalten Injection mit Kleistermasse, ibid., 1880, p. 232.

10. Bianchi, S., quoted by Spalteholz. Cohnheim and von Schulthess-Rechberg: Ueber die Folgen der Kranzarterienverschliessung für des Herz, Virchow's Arch. f. path. Anat. **85**:503, 1881. Erdös, Johann: Eine Methode zur Injektion der Blutgefässe mit kaltflüssiger Masse, Anat. Anz. **3**:261, 1888.

11. Müller (footnote 6, third reference).

12. Dragneff, Stéphane: Récherches anatomiques sur les artères coronaries du coeur chez l'homme, Bibliog. Anatomique **4**:111, 1896. Teichmann, L.: Injection-Mass, J. Roy. Micr. Soc. **2**:125 and 716, 1882.

13. Kollmann, J.: Teichmann's Cold Injection, J. Roy. Micr. Soc. **15**:704, 1895.

14. Budge, Albrecht: Die Saftbahnen im hyalinen Knorpel, Arch. f. mikr. Anat. **14**:65, 1877.

15. Frank, F., quoted by Mayer (footnote 4).

16. Erdös (footnote 10, third reference).

17. Jachtchinsky, S.: Sur l'emploi du silicate de soude (verre liquide) comme substance à injector pour les préparations macroscopiques du système vasculaire, Anat. Anz. **24**:204, 1903. Roger, quoted by Unna (footnote 7, second reference).

(dissolved in alcohol), thymol [20] (Wywodzen's mass) and mercury.[21] No matter which substance was used, without further modifications the method could never be of much value except in microscopic work.

Serial sections are valuable for more minute work. Reconstructions have been made from sections,[22] but obviously they can only serve for the study of limited areas.

Methods of silver impregnation used by Hoyer and Unna depend on the fact that silver nitrate, when injected into the blood vessels or applied to sections of tissue, will deposit silver granules in the endothelial cells. This technic has proved of great value in the study of the

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18. Taguchi, quoted by Unna (footnote 7, second reference).

19. Hoyer, H.: Beiträge zur anatomischen und histologischen Technik, Arch. f. mikr. Anat. **13**:645, 1877. Schuberg: Zur Injektionstechnik, Zool. Anz. **16**:142, 1893.

20. Wywodzen, D.: Wywodzen's Injecting Material, J. Roy. Micr. Soc. **2**:717, 1882.

21. Nuck, quoted by Brühl (footnote 48).

22. Gross, Louis: The Blood Supply to the Heart, New York, Paul B. Hoeber, 1921, p. 152.

23. Haschek, E., and Lindenthal, O. T.: Ein Beitrag zur praktischen Verwerthung der Photographie nach Röntgen, Wien. klin. Wchnschr. **9**:63, 1896.

24. Dutto, Uberto: Photographies du système artériel obtenues avec les rayons de Röntgen, Arch. ital. de biol. **25**:320, 1896.

25. Braus, Hermann: Ueber Photogramme von Metallinjection mittelst Röntgen-Strahlen, Anat. Anz. **11**:625, 1896. Esar, Paul: Intravitale füllung des Gefässystems mit Kontrastmitteln, Klin. Wchnschr. **3**:280, 1924. Fryett, A. G.: Stereoscopic Plates of the Heart, Showing Injected Coronary Arteries, Arch. Roentg. Ray. **10**:206, 1905; Radiograph of Heart, Coronary Vein Injected, ibid., p. 236. Glew, F. H.: Radiogram of the Arterial System of an Injected Infant, ibid. **4**:24, 1899. Leopold and Leisewitz, quoted by Vogt (footnote 32). Musgrove, C., quoted by Ferguson (footnote 35). Oberhelman, H. A., and LeCount, E. R.: Variations in the Anastomosis of the Coronary Arteries and their Sequences, J. A. M. A. **82**:1321 (April 26) 1924. Stiles, H. J., quoted by Hinman, Morison and Lee-Brown (footnote 3, second reference).

26. Delkeskamp, Gustav: Das Verhalten der Knochenarterien bei Knochenerkrankungen und Frakturen, Fortschr. a. d. Geb. d. Röntgenstrahlen **10**:219, 1906-1907. Fraenkel, E.: Ueber die Blutgefässversorgung des Wurmfortsatzes, ibid. **9**:1, 1905-1906. Hildebrand: Ueber die Methode, durch Einbringen von schattengebenden Flüssigkeiten Hohlorgane des Körpers im Röntgenogramm sichtbar zu machen, ibid. **11**:96, 1907. Lexer, E.: Die Entstehung entzündlicher Knochenherde und ihre Beziehung zu den Arterienverzweigungen der Knochen, Arch. f. klin. Chir. **71**:1, 1903. Opitz: Drei Aktinogramme von einem Arteriosklerotiker und einem mit graue Salbe injizierten Präparate, Fortschr. a. d. Geb. d. Röntgenstrahlen. **1**:70, 1897. Parker, G. H.: Notes on Roentgen-Ray Injection Masses, Anat. Record **7**:247, 1913.

27. Hewson, A., quoted by Gough (footnote 28, first reference). Legueu, Felix, Papin, Edmond, and Maingot, Georges: Exploration radiographique de l'appareil urinaire, Paris Soc. d'éd. scient. et med. **8**:243, 1913.

smaller vessels and the capillaries, particularly in microscopic sections. However, its field of usefulness must be limited to specialized researches.

The injection of an opaque material followed by roentgenography, because of the ease and rapidity with which it can be employed, has been used extensively in the study of the circulation in general, and especially in that of the blood supply to the heart. In 1896, shortly after the introduction of the roentgen ray, Teichmann's mass [23] and calcium sulphate [24] were each used to inject the blood vessels, and satisfactory roentgenograms were obtained. Since that time, many other opaque injection masses have been utilized. These include mercury alone,[25] mixed with other substances,[26] mercury salts,[27] the salts of barium,[28] bismuth [29] and calcium,[30] colloidal silver,[31] red lead,[32] lead

28. Gough, J. A.: A Method of Injecting the Blood-Vessels for Roentgenological Studies and Simultaneously Embalming, Anat. Rec. **18**:193, 1920. Gross, Louis: Studies on the Circulation of the Kidney in Relation to Architecture and Function of the Organ in Health and Disease, J. M. Research **36**:327, 1917; footnote 22. Hinman, Morison and Lee-Brown (footnote 3, second reference). Smith, F. M.: Concerning the Anatomy of the Coronary Arteries, Am. J. M. Sc. **156**:706, 1918.

29. Baumgarten, W.: Infarction in the Heart, Am. J. Physiol. **2**:243, 1899. Beck, E. G.: The Stereoscopic Radiograph as an Aid to the Surgeon, Surg. Gynec. Obst. **12**:1, 1911. Liek, E.: Ein weiterer experimenteller Beitrag zur Frage des arteriellen Collateralkreislauf der Niere, Arch. f. klin. Chir. **106**:435, 1915. Camphbell, J., and Pennefather, C. M.: An Investigation into the Blood-Supply of Muscles with Special Reference to War Surgery, Lancet **1**:294, 1919. Katzenstein, M.: Ueber die Möglichkeit der Ausbildung eines arteriellen Collateralkreislaufes der Niere, Berl. Klin. Wchnschr. **48**: 1651, 1911. McPhedran, F. M., and Weyl, C. N.; Clinical and Physiologic Deductions from Synchronized Roentgen-Ray Exposures, Am. J. M. Sc. **173**:313, 1927. Parker (footnote 26, sixth reference). Sampson, J. A.: The Blood Supply of Uterine Myomata, Surg. Gynec. Obst. **14**:215, 1912. Stegman, R.: Ersatz des extirpierten Calcaneus durch Drehung des Talus, Fortschr. a. d. Geb. d. Röntgenstrahlen **9**:396, 1905-1906. Terry, W. I.: Radium Emanations in Exophthalmic Goiter: Blood Vessels of Adenomas of the Thyroid, J. A. M. A. **79**:1 (July 1) 1922; footnote 4, third reference.

30. Preiser, Georg: Eine typische posttraumatische und zur Spontanfraktur führende Ostitis des Naviculare Carpi, Fortschr. a. d. Geb. d. Röntgenstrahlen. **15**:189, 1910. Dutto (footnote 24).

31. Franck, O., and Alwens, W.: Kreislaufstudien am Röntgenschirm, München. med. Wchnschr. **57**:950, 1910. Skinner, E. H.: Circulatory Opacity, Am. J. Roentgenol. **4**:75, 1912.

32. Descomps, P.; de Fallestans, G., and de Lalaubie, G.: Technique pratique pour injections et radiographies de pièces anatomique, Bull. et mém. Soc. anat. de Paris **85**:493, 1910. Amenomiya (footnote 6, first reference). Hauch: Die Arterien der gesunden und kranken Niere im Röntgenbilde, Fortschr. a. d. Geb. d. Röntgenstrahlen **20**:172, 1913. Jamin, F., and Merkel, H.: Stereoscopic Skiagrams of the Coronary Arteries of the Human Heart under Normal and Pathologic Conditions, abstr., Arch. Roentg. Ray. **11**:328, 1906-1907. Preiser (footnote 30, first reference). Vogt, E.: Röntgenuntersuchungen über die Arterien der normalen placenta, Fortschr. a. d. Geb. d. Röntgenstrahlen. **21**:30, 1914.

chromate,[33] chrome yellow,[34] vermilion,[35] sodium bromide [36] and iodized oils.[37] Pictures obtained in this manner, particularly stereoscopic, have been valuable in the visualization of circulatory networks, and this method is the only one now known by which one can hope to study the blood vessels of man during life. Woollard [38] stated:

> By injection the x-ray pictures confirm the results already discovered by Spalteholz, for example, of the existence of anastomosis of the vessels of the heart. On the whole, not much additional information has been obtained in this way. Other methods, such as Spalteholz's clearing method, have been more useful.

In 1907, Spalteholz [39] introduced a new method of studying the coronary circulation. He injected both coronary arteries with chrome yellow gelatin, fixed the heart in 10 per cent solution of formaldehyde, bleached it with hydrogen peroxide, washed it in water, dehydrated it in alcohol and placed it first in benzene, then in a mixture of oil of wintergreen and benzyl benzoate and finally in a special tank to remove all of the air and benzene. By this technic, the walls of the heart were cleared and rendered semitransparent, and as the injected vessels remained opaque, they could be readily traced through the cleared muscular structures. This method is the one by which most of the recent advances in the knowledge of the coronary circulation, such as the existence of anastomosis, the blood supply to the mitral valve and the blood supply to the conduction system have been worked out. The obvious reason why this method has proved superior to the roentgenographic method is that the former preserves the relationship of the blood vessels to the parts which they supply, while this relationship is lost in the stereoscopic roentgenogram, even though it may show a fairly complete picture of the entire vascular tree. Spalteholz's

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33. Parker (footnote 26, sixth reference).

34. Spalteholz, W., and Hirsch, C.: Coronararerien und Herzmuskel: Anatomische und experimentelle Untersuchungen, Deutsche. med. Wchnschr. **1**:790, 1907. Spalteholz, W.: Die Arterien der Herzwand, Leipzig, 1924, p. 165.

35. Ferguson, F. R.: Roentgenological Injection Masses—Old and New, J. Anat. **59**:297, 1924-1925. Hauch (footnote 32, third reference). Raw, N., quoted by Ferguson.

36. Hinman, Morison and Lee-Brown (footnote 3, second reference).

37. Cadenat, F. M., and Py: Endartérite oblitérante du tronc axillo-huméral droit; Repérage du siège de l'oblitération par injection intra-artérielle de lipoidol; Extraction du caillot sans résultat, Bull. et mém. Nat. Soc. de chir. de Paris **50**: 1149, 1924. Coffey, W. B.: Angina Pectoris, ed. 1, New Orleans, A. J. Dickinson, 1927, p. 104. Csapody, von J.: Arterienphotographien vermittels Lipiodol, Klin. Wchnschr. **4**:2327, 1925. Rouslacroix: Exploration des artères coronaires du coeur par l'injection lipiodelée, Compt. rend. Soc. de biol. **93**:1446, 1925.

38. Woollard, H.: Recent Advances in Anatomy, Philadelphia, P. Blakiston's Son & Company, 1927, vol. 7, p. 290.

39. Spalteholz (footnote 34, first reference).

method,[40] however, in spite of modifications which have been made by Bayne-Jones,[41] Tandler,[42] Gross[43] and Koch[44] has some definite limitations. The clearing is a slow process, and it is generally far from complete even in the smaller hearts, while in the larger ones the left ventricle is seldom rendered entirely transparent so that the picture of the coronary circulation presented is usually incomplete.

In many respects, the most important method is that of injections followed by corrosion or maceration. The corrosion is accomplished by allowing the injected preparations to remain in alkaline or strong acid solutions; the maceration is accomplished by decomposition with larvae and putrefactive bacteria.

Swammerdamm[45] (1672), according to Unna, injected hot wax into the coarser blood vessels. He was also cited by Brühl who said that he was the first to inject a mass which would become hard.

Bidloo[46] (1685) used Rose's metal (vide infra) for purposes of injection. After the injection, he boiled the parts to decompose the tissues surrounding the vessels.

Ruysch[47] (1704) forced a metal of secret composition into the coronary vessels and then allowed larvae and maggots to attack and disintegrate the muscle tissue. His description of the coronary arteries has not met with the approval of later writers. Unna, for example, agreed with Hyrtl that the injections made by Ruysch were, in the main, extravasations and consequently inaccurate.

Brühl[48] said that Lieberkühn[49] (1711-1746) was the first to use true corrosion. He used Swammerdamm's mass (wax, mixed with fats, and stained with cinnabar) and corroded the parenchyma with nitric acid. Swammerdamm's mass, according to Unna,[50] was also used by Alexander Monro (1730), Schacher (1710), Fabricius (1741), Cassebohm (1740), Barth (1773) and Prochaska (1810).

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40. Spalteholz (footnote 34, second reference).

41. Bayne-Jones, S.: The Blood Vessels of the Heart Valves, Am. J. Anat. **21**:449, 1917.

42. Tandler, J.: Anatomie des Herzens, in Karl von Bardeleben's Handbuch der Anatomie des Menschen, Jena, Gustav Fischer, 1913, vol. 3, pt. 1, p. 292.

43. Gross (footnote 22); (footnote 28, second reference).

44. Koch, Walter: Ueber die Blutversorgung des Sinusknotens und Etwaige Beziehungen des letzteren zum Atrioventrikularknoten, München. med. Wchnschr. **56**:2362, 1909.

45. Swammerdamm (footnote 8, second reference).

46. Bidloo, quoted by Brühl (footnote 48).

47. Ruysch, F., quoted by Spalteholz and others.

48. Brühl, Gustav: Die anatomischen Darstellungsweisen der Hohlräume des Ohres und der Nase, Anat. Anz. **14**:418, 1898.

49. Lieberkühn, quoted by Brühl (footnote 48).

50. Unna (footnote 7, second reference).

It is to Hyrtl [51] (1855), however, that credit for most of the development of corrosion technic is due. Brühl called him the master of corrosion. He used various metals of a low melting point but did most of his work with wax, mixing one part of it with six parts of resin. The actual corrosion was done with hydrochloric acid.

Hoyer [52] (1877) found that solutions of shellac thinned with alcohol made the best injection masses for gross and fine vessels. He claimed that this mass could be used in making corrosion preparations and advised the addition of cinnabar as a coloring material when it is used for this purpose.

Altmann [53] (1879) injected blood vessels with olive oil, treated the part with osmic acid, froze it and then placed it in Javelle water [54] for corrosion. Only small sections could be used by this method. The osmic acid hardened the olive oil in the vessels and, since fats are more resistant to acids, they were not as quickly corroded as the tissues. The time necessary for corrosion varied from a few minutes to several hours, and if the action was continued too long the injection mass crumbled. Consequently, this method has not been widely adopted.

Müller [55] (1906) utilized a metal of low melting point, and Flint [56] used Wood's metal and celloidin for studying the structure of the lungs. Ten per cent potassium hydroxide was used for corrosion.

Lipowitz's metallic alloy of low melting point (from 65 to 70 C.), containing cadmium, tin, bismuth and lead, was employed by Nussbaum [57] (1912) for injections in the coronary circulation. Since hydrochloric acid attacked this alloy, he found it necessary to use 15 per cent potassium hydroxide to remove the soft parts. His preparations for corrosion of the coronary circulation are the best that have ever been obtained with metals.

Ghoreyeb [58] (1916) injected the coronary arteries with Wood's metal in order to determine their capacity. Corrosion was carried out in order to recover the metal.

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51. Hyrtl, quoted by Brühl (footnote 48); quoted by Spalteholz.

52. Hoyer (footnote 19, first reference).

53. Altmann, R.: Ueber die Verwerthbarkeit der Corrosion in der mikroskopischen Anatomie, Arch. f. mikr. Anat. **16**:471, 1879.

54. A solution of sodium hypochlorite prepared by adding soda ash to a solution of calcium hypochlorite.

55. Müller, Josef: Zur vergleichenden Histologie der Lungen unserer Haussäugetiere, Arch. f. mikr. Anat. **69**:1, 1906.

56. Flint, J. M.: The Development of the Lungs, Am. J. Anat. **6**:1, 1906-1907.

57. Nussbaum, Adolf: Ueber das Gefässsystem des Herzens, Arch. f. mikr. Anat. **80**:450, 1912.

58. Ghoreyeb, A. A.: Studies on the Circulation: I. The Effect of Disease on the Renal Arterial Bed; II. The Capacity of the Coronary Arteries as Related to Weight and Age of the Heart, J. M. Research **35**:87, 1916-1917.

The injection of a celloidin or celluloid mass into the blood vessels, followed by corrosion of the tissues, has been used for many years and by various investigators. It was first employed by Schiefferdecker [59] (1882), who prepared a mass of celloidin, dissolved in concentrated alcohol and ether, and colored by asphaltum, Berlin blue or cinnabar. Digestion was performed by hydrochloric acid. Hochstetter [60] (1886) claimed that he began using celloidin at the same time, but independently of Schiefferdecker. He recommended the addition of kaolin because of the shrinkage of the casts obtained by Schiefferdecker's method. His masses were colored with cobalt blue, chrome yellow or cinnabar. Storch [61] (1898) made three distinct improvements in the celloidin technic: (1) the employment of acetone as a celloidin solvent; (2) the injection of a thicker mass (after a preliminary injection of a thin one) to insure a firm cast of the larger trunks, and (3) the prevention of shrinkage by the substitution of celluloid for celloidin. Peabody [62] (1897) used Prussian blue as a coloring agent in the injection of the lateral line canals in the smooth dogfish. Brödel [63] (1901) claimed to have used Schiefferdecker's corrosion method as modified by Mixter and Mall,[64] employing an alcohol and ether solution of celloidin. Digestion was performed by pepsin dissolved in hydrochloric acid.

Flint [65] (1901) used both commercial celluloid and a mixture of celloidin [66] and camphor dissolved in acetone. The addition of camphor was a definite improvement, for it prevented the shrinkage of the casts and made it unnecessary to preserve them in glycerin. Flint recommended corrosion of the tissues in concentrated hydrochloric acid

59. Schiefferdecker, P.: Ueber die Verwendung des Celloidins in der anatomischen Technik, Arch. f. Anat. u. Entwcklngsgesch, 1882, p. 199.

60. Hochstetter, Ferdinand: Ueber eine Modifikation der Schiefferdeckerschen Celloidinkorrosionsmasse, Anat. Anz. **1**:51, 1886.

61. Storch, Carl: Das Celloloid und seine Anwendung zur Injektion von Blutgefässen, Ztschr. f. Tiermed. **3**:173, 1898.

62. Peabody: The Ampullae of Lorenzini of the Selachii, Zoöl. Bull., Boston **1**:163, 1897-1898.

63. Brödel, Max: The Intrinsic Blood Vessels of the Kidney and Their Significance in Nephrotomy, Bull. Johns Hopkins Hosp. **12**:10, 1901.

64. Mall, F. P.: A Study of the Structural Unit of the Liver, Am. J. Anat. **5**:228, 1906.

65. Flint, J. M.: The Ducts of the Human Submaxillary Gland, Am. J. Anat. **1**:269, 1901-1902.

66. Celloidin is a concentrated preparation of pyroxylin. Celluloid is essentially pyroxylin and camphor. Satisfactory preparations are not made with celloidin unless camphor is added. Since the addition of camphor to celloidin forms celluloid, it would seem more proper to speak of those preparations containing both celloidin and camphor as celluloid injection masses, even though this does not conform to general usage.

rather than by the slower method of digestion by pepsin. Krassuskaja [67] (1904) also used a mass of photoxylin (or celloidin) and camphor in acetone.

In 1906, Huber,[68] after having experimented with various dyes, introduced alkanin, a red pigment, which is soluble in acetone and can be used for the smallest vessels without causing crumbling of the preparations. He introduced a constant pressure device by attaching the cannulas by rubber tubing to a tank of compressed air. He found that a sudden release of a high pressure (from 20 to 25 pounds [from 9 to 11 Kg.]) at the onset was necessary in the injection of the finer vessels, and suggested a stock solution of the injection mass containing 30 Gm. of celloidin and 20 Gm. of camphor dissolved in 600 cc. of acetone. Marshall [69] (1923) mixed 10 per cent solutions of crystal violet and brilliant green to make a permanent blue for celluloid preparations. Morison [70] (1923) found that old roentgen-ray films could be used as a cheaper means of obtaining celluloid, but considered them satisfactory only in the preparation of coarser specimens. He recommended the substitution of a more concentrated solution of the mass after the injection had been running for ten or fifteen minutes in order to obtain a solid cast of the larger vessels. The casts were kept in a preservative solution containing 100 cc. of water, 2 cc. of solution of formaldehyde and 20 cc. of glycerin. Terry and Delamere [71] (1924) used this technic in studying the circulation of adenomas of the thyroid gland.

Counseller and McIndoe [72] (1926) forced cold water through the vessels of the liver to remove the blood and bile. Weights were then placed on the specimens, and they were wrapped in towels to remove as much of the fluid as possible. Their other modifications were the utilization of artist's oil paints for coloring, the lengthening to two days of the time that the injection was continued under constant pressure and the adoption of a dry method of mounting the corrosion preparations, using the technic described by Lundquist and Robertson.[73] Barker [74] (1927) followed the technic described by Counsellor and

67. Krassuskaja, A.: Abstr. by Stieda, Anat. Hefte **13**:521, 1904.

68. Huber, G. C.: The Arteriolae Rectae of the Mammalian Kidney, Am. J. Anat. **6**:392, 1906-1907.

69. Marshall, J. A.: Method for Preparing Blue Celluloid Injection Material, J. A. M. A. **80**:181 (Jan. 30) 1923.

70. Morison (footnote 3, second reference).

71. Terry and Delamere (footnote 4, third reference).

72. Counseller, V. S., and McIndoe, A. H.: Dilatation of the Bile Ducts (Hydrohepatosis), Surg. Gynec. Obst. **43**:729, 1926.

73. Lundquist, L. R., and Robertson, H. E.: The Dry Mounting Method for Museum Specimens, Hosp. Prog. **7**:273, 1926.

74. Barker, N.: Personal communication to the author.

McIndoe, except that he increased the time of injection from two to five days or even longer. Scott and Moore [75] (1928) believed that any homogeneous mass with a relative viscosity of 18.5 (as compared to water) is sufficient for injection of the renal circulation to, but not beyond, the efferent glomerular arteries. For celloidin, they found that a solution of this viscosity contained 2.75 per cent celloidin and 2 per cent camphor dissolved in acetone.

Although celluloid and celloidin have been used as injection masses for a considerable length of time, I have not been able to find more than three references to their use in the study of the coronary vessels. Kerr and Mettier [76] (1925) published two photographs of injections of celloidin in the coronary arteries in man made by Faulkner and Epstein, but they failed to show relationships because a cast was not made of the interior of the heart. Spalteholz [77] discouraged the use of celloidin, and his influence has probably done much to keep this material from being used more extensively as an injection mass of the coronary vessels. Quite recently (1928) Wearn [78] employed thick masses of celloidin as well as wax in studying the thebesian vessels. Corrosion was not attempted.

In demonstrating the coronary circulation, a cast of the interior of the heart should be made; otherwise, the parts supplied by each vessel remain obscure. It is surprising that so little work of this nature has been undertaken. Brühl, in discussing corrosion preparations of the cavities in and around the ear and nose, mentioned the following materials that have been employed: (1) wax resin; (2) paraffin; (3) metals of low melting point, including Arcet's metal (one part lead, two parts tin, two parts bismuth), Rose's metal (one part lead, one part tin, two parts bismuth) and Wood's metal (eight parts lead, four parts tin, sixteen parts bismuth, three parts cadmium) and (4) celloidin.

The first two substances have been utilized in the measurement of the capacity of the chambers of the heart. Periot [79] and Gaston [80] (1925) made plaster-of-paris casts of the left ventricle. They cited

75. Scott, Ernest, and Moore, R. A.: Vascular Injection in Pathology, J. Lab. & Clin. Med. **13**:481, 1928.

76. Kerr, W. J., and Mettier: The Circulation of the Heart Valves: Notes on the Embolic Basis for Endocarditis, Am. Heart J. **1**:96, 1925-1926.

77. Spalteholz, W.: Die Arterien der Herzwand, text and footnote, 1924, p. 5.

78. Wearn, J. T.: The Rôle of the Thebesian Vessels in the Circulation of the Heart, J. Exper. Med. **47**:293, 1928.

79. Periot, M.: Casts of the Interior of Heart Cavities, Compt. rend. Soc. de biol. **92**:615, 1925.

80. Gaston: Un procédé d'étude de la morphologie des cavités cardiaques, Compt. rend. Soc. de biol. **92**:614, 1925.

Collin, Hiffelsheim and Robin [81] (1864) and Tandler [42] as having made casts of the cardiac cavities previous to their work and stated that, except for them, investigators had not used casts in determining the size of these cavities. Tandler published photographs of casts of the interior of the heart in his work on anatomy. According to Gaston, all these observers poured melted wax or paraffin into the heart.

Nussbaum [57] (1912) seems to have been the only one to make casts of the cardiac cavities with metals. He filled the heart with paraffin. Eight days later, he melted out the paraffin and replaced it with Lipowitz metal, which was similar to the other metals of low melting point, containing three parts of cadmium, four parts of tin, eight parts of lead and fifteen parts of bismuth; this metal had the same melting point as Wood's metal (from 65 to 70 C.) and was identical with that substance except that it contained one less portion of bismuth. Excellent casts of the heart and of the coronary vessels were obtained.

Although celluloid and celloidin have been used in the pelvis of the kidney (by Morison,[70] Barker [74] and others) and in the cavities in and about the nose and ear,[82] I have not found any reference to their use in the molding of casts of the cardiac cavities. In fact, I have found no reference to their use in the making of casts of any structure as large as the interior of the adult heart. It seems advisable, therefore, to publish the modifications of the celluloid technic which have been found necessary for the injection of the cardiac cavities.

A MODIFICATION OF THE CELLULOID AND CORROSION TECHNIC

Apparatus.—The apparatus which I have employed is almost identical with that used by Counseller and McIndoe in making injections in the bile ducts of the liver (fig. 1). Constant air pressure is used instead of the gas which is shown in the diagram. The mercury manometer aids in adjusting the pressure accurately. A second mercury manometer is also needed, as a lower pressure is used in injections in the interior of the heart. Three of the small bottles for the injection mass are needed for each heart in which injection is to be made. By means of Y-tubes and additional connections of rubber tubing, injections can be performed in any number of hearts at one time. The hearts are kept in a bath of running, cold water while the injection is in progress. This delays disintegration and aids in dissolving the acetone from the injection mass.

81. Collin, Hiffelsheim and Robin, quoted by Periot (footnote 79) and Gaston (footnote 80).

82. Siebenmann, quoted by Brühl (footnote 48). Steinbrüge, H.: Zur Corrosions-Anatomie des Ohres, Centralbl. f. d. med. Wissensch. **23**:545, 1885.

Stock Solution.—The injection mass is prepared in a concentrated form. The constituents of the stock solution, which is the same as Morison's solution C, are as follows: 100 Gm. old roentgen-ray films; 80 Gm. pure gum camphor, and 1,000 cc. of acetone. The emulsion is scrubbed off the films in warm water, and the films are cut into small pieces so they will dissolve more readily in the acetone. The stock bottle should be inverted every day so the films may become dissolved more rapidly. The mechanical stirring and the filtration described by

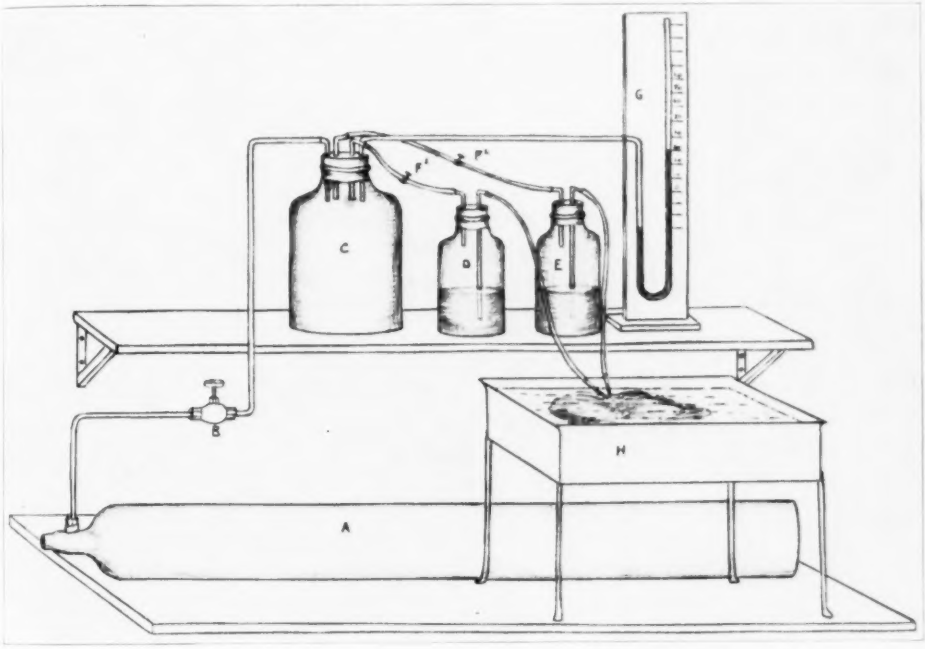

Fig. 1.—Injection apparatus (from Counsellor and McIndoe). *A*, gas tank; *B*, needle valve; *C*, lead bottle; *D*, injection bottle; *E*, injection bottle; F^1, F^2 stopcocks; *G*, mercury manometer; *H*, specimen tank.

Morison have not been found necessary. In five or six days the mass is ready to use. The dilution of the stock solution for purposes of injection is discussed elsewhere. Artist's oil paints, as recommended by Counseller and McIndoe, have proved satisfactory coloring materials. The paints must be well mixed in the solution immediately before the injection is started. As a routine I have used Prussian blue for the right coronary artery, vermilion for the left and silver white for the veins and the casts of the heart chambers.

Preparation of the Specimen.—Injections are much more easily performed in the heart and vessels if care is taken in the removal of the specimen at necropsy. The heart and lungs, together with that portion of the diaphragm which surrounds the inferior vena cava, are removed en masse. Then the heart and great vessels are dissected free from the lungs and diaphragm, care being taken to have the stumps of the great vessels as long as possible. This necessitates cutting the pulmonary vessels close to the lungs.

The chambers of the heart should be washed thoroughly with water. The water must be forced through in the direction of the blood flow of the heart; that is, the stream must be directed into either the superior or the inferior vena cava to wash out the right side and into one of the pulmonary veins to remove the blood clots from the left side of the heart. A massage of the heart will aid in removing some of the clots. Others will have to be extracted with forceps. All must be removed if a perfect cast is to be obtained.

Technic of Arterial Injections.—The injection of the arteries is not particularly difficult and is not unlike the injection of the portal vessels or the renal arteries, which has been well described by others.[83] The aorta must be cut down longitudinally toward the heart until the coronary arteries are seen, but not so far that it would be impossible to tie off the vessel later. Then the cannulas must be inserted through the orifices of the coronary arteries and tied firmly in place. In order that this may be done, the first part of each coronary artery must have been freed previously, and ligatures passed beneath the vessels. The ligatures (two or three for each cannula) are then tied firmly and so tight that the cannula will not slip if twisted slightly. The cannulas must be inserted when the heart is quite fresh; otherwise they or the ligatures are likely to cut through the vessels.

The coronary arteries are each washed with water at a pressure of 250 mm. of mercury until the water is no longer blood-tinged. An effort is not made to remove the water from the vessels before the injection is started, since water hardens the mass. Although the arterial injections are not started until the veins and the chambers of the heart have become distended with the mass, they will be commented on first for the sake of simplicity.

A 1:4 or 1:5 dilution of the stock solution (one part of the stock solution to four or five parts of acetone) is generally used for the arterial injection. This dilution assures the injection of the smaller vessels. Artist's oil paint is added to color the solutions, vermilion being used for the left coronary artery and Prussian blue for the right.

83. Counseller and McIndoe (footnote 72). Hildebrand (footnote 26, third reference).

The celluloid mass, after being well mixed, is injected into both coronary arteries simultaneously at an initial pressure of about 450 mm. of mercury, and is maintained at this pressure for an hour or two, after which the pressure is reduced to one ranging from 150 to 250 mm. at which the injection is continued for from five to seven days. To strengthen the larger trunks, a more concentrated solution (1:2 or 1:3 dilution of the stock solution) should be substituted within the first four hours. If the injection is not continued for from five to seven days, the larger vessels are likely to be hollow and consequently fragile.

Technic for Molding Casts of the Cavities and the Veins.—After the cavities of the heart have been washed free of blood clots, the foramen ovale is opened, by enlarging the opening, if patent, or by passing the points of blunt scissors or forceps through the thinnest portion of the interauricular septum (through the fossa ovalis) and spreading the points of the instrument until the opening is adequate. If this is not done there will not be a point of union between the casts of the two sides of the heart, and the preparation will fall apart after corrosion. One wide-tipped glass cannula is placed in the orifice of either the superior or the inferior vena cava, and another is placed in the opening of one of the pulmonary veins. Both cannulas are tied securely. All the other great vessels are then tied successively. It is rather difficult to ligate the aorta owing to the two cannulas (one to each coronary artery) within it. This can be accomplished, however, so that there is not any leakage, if the aorta has been divided longitudinally, each half being wrapped around the adjacent cannula and tied securely in several places, and finally the two portions of the aorta being tied firmly together. If any of the great vessels have been cut too short, the opening can be closed by inserting a tightly fitting cork into the lumen, the large end inward, and tying the stump of the vessel over the cork. When every orifice is closed, rubber tubing is attached to the cannula in the vena cava and to the one in the pulmonary vein. The tubing to the latter may be clamped, as generally the left side of the heart will fill from the right. If, for any reason, the left side of the heart does not fill, and this occasionally happens, celluloid may readily be injected through the otherwise unused cannula into the pulmonary vein.

The celluloid mass is forced into the cavities of the heart with the same apparatus with which injections are made in the arteries. When it is desired to make an injection in the veins of the heart, a thin solution should be used first. For this purpose, a solution of one part of the stock solution to three or four parts of acetone, to which artist's oil paint (white) has been added for coloring, is used. This solution must be stirred until it is well mixed. After 500 cc. has been emptied

into the heart, a thick solution is substituted. The stock solution may be used for this purpose, but the mass generally flows in a little more readily if it is thinned slightly with acetone. An effort must be made to tie off all leaks that may occur when the celluloid is injected. The smaller leaks will be closed by the injection of a thicker celluloid mass. I attempt to use continuous injection at a pressure of from 20 to 60 mm. of mercury in filling the cavities, but if leakage occurs this may not be possible at the onset. When leakage takes place, pressures ranging from 60 to 120 mm. of mercury are used, but only long enough to allow the celluloid to flow in until the auricles are rendered slightly tense to palpation. The pressure is then clamped off and the process repeated about every half hour during the first day and about every one to three hours thereafter. When there is no leakage, the injection is continued until soft areas cannot be palpated on the surface of the auricles and until the cannulas are firmly cemented in. A uniform cast rather than a solid cast is desirable; hence, it is necessary to turn the heart in various positions from time to time as the celluloid tends to become deposited in the dependent portions. It is sometimes necessary to puncture the walls of the auricles with a hypodermic needle and inject water before the mass in the cavities will harden. This allows the air and acetone to escape, makes room for more of the mass to enter and supplies fresh water to remove the acetone and to precipitate the celluloid.

The injection of the veins and of the cast is begun first so that the arteries will not be stretched after the arterial injection has been started. The injection of the arteries is continued uninterruptedly for from five to seven days, and that of the interior of the heart for from five to fourteen days, depending on the size of the heart and on other variable factors.

Corrosion.—When the injection is considered completed, the heart is placed in concentrated hydrochloric acid and allowed to remain there for from five to fifteen days depending on the thickness of the myocardium and the rate of its corrosion.

Washing.—When the corrosion is completed, the heart is immersed in water and a fine stream is directed against it to wash away everything but the cast. It is important to direct this stream so that it strikes just below the surface of the water. This allows the corroded tissues to float away and minimizes the injury to the smaller vessels. The washing does not have to be completed at once; in fact, washing is more easily carried out if the specimen is allowed to stand over night in water. If all the tissues cannot be washed away, the specimen should be placed again in fresh concentrated hydrochloric acid and left there for from twelve to twenty-four hours. Sometimes it is difficult to remove the epicardial fat. This can be remedied by placing the heart in xylene

for a few hours, which will dissolve out the fat without injury to the cast. The heart is then washed again with water to remove the adhering particles of fat and the xylene.

Preservation.—After the washing is completed, the specimen is allowed to stand in water to which enough sodium bicarbonate has been added to neutralize the acid that may still be present. A coating

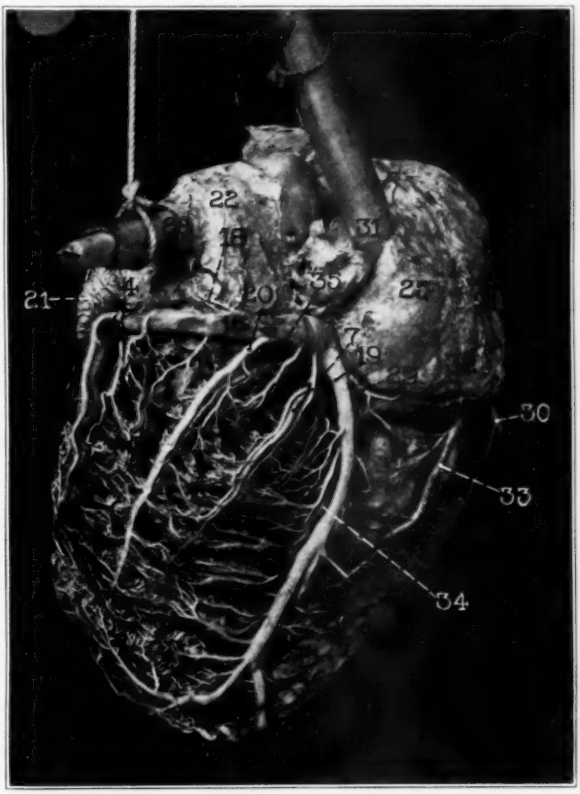

Fig. 2.—Posterior view of celluloid cast of heart from a girl, aged 10 years, who had died from generalized peritonitis; *21*, left auricular appendage; *22*, left auricle; *25*, right auricle; *31*, inferior vena cava; *28*, left pulmonary veins; *4*, left circumflex artery; *32*, right coronary artery; *33*, posterior artery of right ventricle; *34*, posterior descending artery; *7*, posterior artery of left ventricle; *35*, accessory posterior artery of left ventricle; *19*, vein of posterior interventricular sulcus; *29*, right coronary vein; *20*, posterior vein of left ventricle; *16*, coronary sinus; *18*, oblique vein of Marshall; *30*, vein of acute margin.

of varnish is applied with a fine spray from an atomizer; it is better to delay this procedure until all photographs have been taken. The

preparations can be mounted dry according to the method of Lundquist and Robertson, or wet, using the solution of water, formaldehyde and glycerin described by Morison.

The Finished Preparations.—The completed specimen shows the left coronary artery injected in red, the right coronary artery in blue and the coronary veins in white. Figures 2 to 4 are actual photographs of specimens in which injections were made. The wall of the heart has

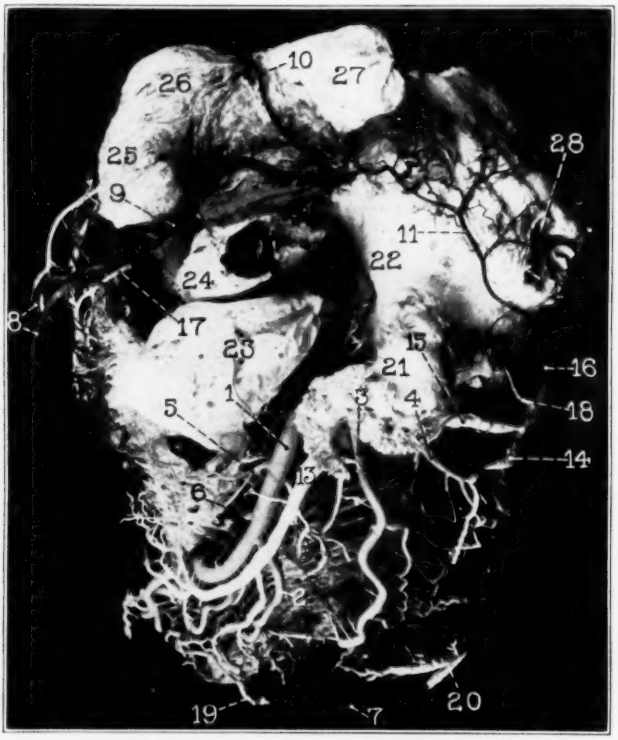

Fig. 3.—Anterosuperior view of celluloid cast of heart of woman, aged 44, who died from carcinoma of the rectum; *21*, left auricular appendage; *22*, left auricle; *23*, pulmonary artery; *24*, aorta; *25*, right auricular appendage; *26*, right auricle; *27*, superior vena cava; *28*, left pulmonary veins; *1*, anterior descending artery; *2*, first accessory anterior descending artery; *3*, second accessory anterior descending artery; *4*, circumflex artery; *5*, artery to conus arteriosus; *6*, artery to interventricular septum; *7*, posterior artery of left ventricle; *8*, anterior arteries of right ventricle; *9*, anterior artery of right auricle; *10*, artery of crista terminalis (artery to sinus node); *11*, intermediate artery of left auricle; *13*, vein of anterior interventricular sulcus; *14*, vein of obtuse margin; *15*, left coronary vein; *16*, coronary sinus; *17*, anterior vein of right ventricle; *18*, oblique vein of Marshall; *19*, vein of posterior interventricular sulcus; *20*, posterior vein of left ventricle.

been corroded, and the background is the white cast of the chambers of the heart which accurately depicts the internal surface of the heart and thus preserves the normal relations of the coronary vessels, although none of the structures of the living heart remains. All four cavities of the heart have been expanded at the same time, under the same pressure, which is relatively low.

Advantages.—This method of studying the coronary arteries seems to have several distinct advantages over the usual methods, such as

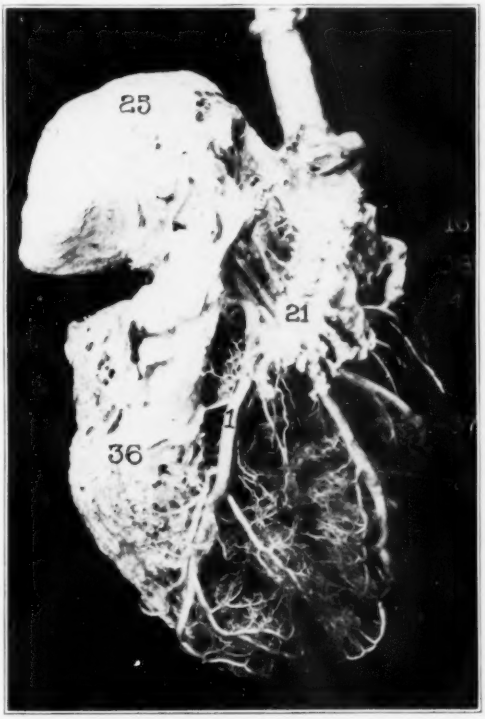

Fig. 4.—Anterior view of celluloid cast of heart of child, aged 13½ months, who died during anesthesia; *25*, right auricle; *36*, right ventricle; *21*, left auricular appendage; *38*, valve of Vieussens; *1*, anterior descending artery; *2*, first accessory anterior descending artery; *37*, second accessory anterior descending artery; *4*, circumflex; *16*, coronary sinus.

injection of opaque materials followed by the taking of roentgenograms, clearing with oil of wintergreen or glycerin, or corrosion without molding a cast of the heart. A cold injection mass is used. The celloidin cast of the interior of the heart shows the size, shape and contour of the chambers, forms a support for the blood vessels and

depicts relationships even after all the tissues of the living heart have been corroded away. The completed injections enable a casual observer to form a fairly accurate impression of the course and relations of the coronary vessels and on that account should be of considerable value in the teaching of anatomy. All structures are in three dimensions; hence, there need not be any confusion in their interpretation, as often occurs with roentgenograms or even stereoroentgenograms. Injections are made in all the veins, whether or not they empty into the coronary sinus. The casts adapt themselves readily to photography. Lastly, celluloid injected into the arteries penetrates to the capillaries but not through them, and, for this reason, the resulting cast is an accurate picture of the arterial tree uncomplicated by venous branches.

Disadvantages.—The chief disadvantage of this method of injection is that it is practically impossible to take microscopic sections of the heart either before or after the injections are made. In addition, while celluloid is flexible, it is nevertheless fragile and must be handled with care, for a broken vessel cannot be replaced.

ATOPY

BLOOD CALCIUM AND GASTRIC ANALYSIS *

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A study of the blood calcium in 167 cases of asthma and allied conditions is presented in the hope of helping to define the importance of calcium determination and calcium treatment in atopic or allergic conditions. The second part of this paper is a preliminary report on the analysis of the gastric contents in fifty cases of atopy.

CALCIUM METABOLISM IN ATOPY

A discussion of this phase of the subject is closely related to the various theories explaining the pathogenesis of asthma and of allied conditions. It is a fairly well established fact that these conditions are due to a form of hypersensitiveness either to some extrinsic protein or to an infection usually of the upper respiratory tract. There are those who insist on the reflex origin of asthma, and somewhat associated with the latter theory is the explanation advanced by Pottenger,[1] who feels that in asthma there is a relative deficiency of calcium ions in the cells of the bronchial system with an imbalance in favor of the potassium ions. This deficiency of calcium results in a lessened irritability of the sympathetic nerve, while the excess of potassium stimulates the parasympathetic or vagus nerve, so that vagotonia and hence bronchospasm and asthma result; therefore, administration of calcium restores the equilibrium, eliminates vagotonia and improves the condition of the patient.

In close relation to these views, a considerable literature is found in which an attempt is made to prove the presence of such a deficiency and the specifice value of calcium therapy. These observations, however, have not gone unchallenged, and it is for this reason that the present study was undertaken. It is necessary to agree as to what constitutes a normal

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* Read before the Society for the Study of Asthma and Allied Conditions, Washington, D.C., April 28, 1928.

1. Pottenger, F. M.: A Discussion of the Etiology of Asthma in its Relationship to the Various Systems Composing the Pulmonary Neurocellular Mechanism with the Physiologic Basis for the Employment of Calcium in its Treatment, Am. J. M. Sc. **167**:203, 1924.

blood calcium. A survey of the literature reveals that from 9 to 11 mg. per hundred cubic centimeters of blood is considered normal. Thus, according to Groves and Vines [2] the average figure is 10.5 mg. According to Halverson,[3] it is 10.2 mg., with a variation of from 9.6 to 10.8 mg. According to Schwartz and Levin,[4] it is from 9 to 12 mg., while Myers [5] finds it to be between 9 and 11 mg., and Caven and Cantarow [6] place it between 6.5 and 9.5 mg. per hundred cubic centimeters of blood. Forty specimens of blood were obtained from normal subjects, medical students, and the blood calcium determined by the use of the Tisdal technic. Twenty-seven of these were obtained on a fasting stomach and thirteen, two hours after breakfast. The lowest reading in the whole series was 8.5 mg., and the highest was 11.7 mg., with an average of 10.43 mg. of calcium per hundred cubic centimeters of blood. It is interesting to note that there was not any appreciable difference in the average value obtained in the fasting blood from the nonfasting blood, the figure for

TABLE 1.—*Blood Calcium in Various Atopic Conditions and in a Normal Person*

	7.5 to 8 Mg.	8 to 8.5 Mg.	8.5 to 9 Mg.	9 to 9.5 Mg.	9.5 to 10 Mg.	10 to 10.5 Mg.	10.5 to 11 Mg.	11 to 11.5 Mg.	11.5 to 12 Mg.	12 to 13 Mg.	Number of Cases	Average Calcium
Asthma	0	4	6	6	16	22	15	6	2	3	80	10.08
Hay-fever	0	0	0	3	5	14	8	6	2	4	43	10.55
Atopic rhinitis	0	1	0	2	2	8	2	3	1	2	21	9.95
Urticaria	2	3	0	3	4	5	3	1	0	0	21	9.66
Angioneurotic edema	0	0	0	0	0	2	0	0	0	0	2	10.4
Normal	0	2	0	2	6	17	11	1	1	0	40	10.43
Total series	2	10	6	16	33	68	39	17	6	9	207	10.19

the former being 10.1 mg., while the latter was 10.34 mg. Table 1 shows that most of the readings were closely grouped.

An analysis was then made of the observations on the blood calcium in 167 cases of atopy, comprising (table 1) eighty cases of asthma, forty-three cases of hay-fever, twenty-one cases of atopic or vasomotor rhinitis, twenty-one cases of urticaria and two cases of angioneurotic

2. Groves, W. R., and Vines, H. W. C.: The Control of Hemorrhage by Intramuscular Injection of Calcium Chloride, Brit. M. J. **2**:40, 1921.

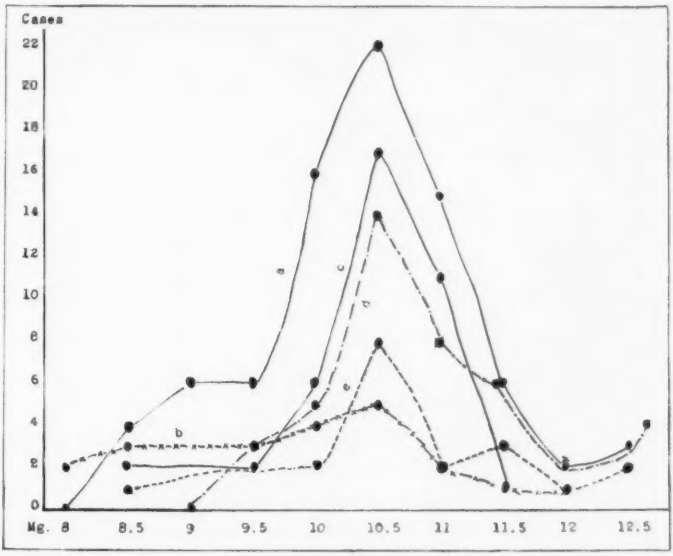
3. Halverson, J. O.; Mohler, H. K., and Bergeim, Olaf: The Calcium Content of the Blood Serum in Certain Pathological Conditions, J. Biol. Chem. **32**: 171. 1917; Calcium in the Blood in Tuberculosis, J. A. M. A. **18**:1309 (May 5) 1917.

4. Schwartz, H. J., and Levin, O. L.: The Calcium Content of the Blood in Various Diseases of the Skin: Based on an Analysis of over 300 Cases, Arch. Dermat. & Syph. **10**:545 (Nov.) 1924.

5. Myers, V. C.: Practical Chemical Analysis of Blood, ed. 2, St. Louis, C. V. Mosby Company, 1924, p. 170.

6. Caven, W. R., and Cantarow, A.: J. Lab. & Clin. Med. **12**:1, 1926; abstr. in J. A. M. A. **88**:126 (Jan. 8) 1927.

edema. The specimens of blood were obtained on a fasting stomach, and the determinations were made according to the technic already mentioned. Thus it is found that the values for the asthma group range between 8.5 and 12.5 mg., with an average of 10.08; in the hay-fever group, between 9.3 and 12.3, with an average of 10.55; in the atopic rhinitis group, between 8.3 and 12.2, with an average of 9.95; in the urticaria cases, between 7.9 and 11.4, with an average of 9.66 and in the two cases of angioneurotic edema, an average of 10.4. The average for the entire 167 cases of atopy is 10.19 mg. per hundred cubic centimeters of blood, as compared with 10.43 mg., the average value found for the



Blood calcium curve in atopy; *a*, asthma; *b*, urticaria; *c*, normal; *d*, hay-fever, and *e*, atopic rhinitis. The similarity in the general trend of the curves in various atopic conditions as compared with the normal should be noted.

forty normal persons. Moreover, most of the cases studied group themselves, as table 1 and the chart show, within a narrow range. It appears, therefore, that a demonstrable calcium deficiency does not obtain in atopic conditions.

This is not in accord with the work of others. Thus, Novak and Hollander [7] agree that a calcium deficiency does not exist in asthma and

7. Novak, F. J., and Hollander, A. R.: Influence of Ultraviolet Irradiation on Calcium Content of Blood Serum in Hay-Fever: Hyperesthetic Rhinitis and Asthma, J. A. M. A. **81**:2003 (Dec. 15) 1923.

in hay-fever, but they find a suggestive lowering in vasomotor rhinitis, their figures ranging from 6.75 to 9.8. Sonnenschein and Pearlman [8] find an average of 10.9 in the same condition, while Brown,[9] considering anything below 9.6 mg. as a definite deficiency, finds such a state to exist in a large percentage of practically all forms of atopy.

What is then the status of calcium therapy in atopy? Calcium has been recommended either alone or in conjunction with either thyroid or parathyroid extract and ultraviolet ray exposure; however, little rational, specific basis seems to exist for such therapy. For, in the first place, since the deficiency of calcium had not been proved to exist, why should calcium be administered? Second, calcium lactate in doses of 5 Gm., three times a day, given together with parathyroid extract for twenty days, as table 2 shows, has apparently little if any influence on the blood calcium [10] of atopic patients. Nor does exposure to ultraviolet rays seem to increase

TABLE 2.—*Effect of Therapy on Calcium Content of Atopic Blood*

Diagnosis	Blood Calcium Before Therapy	Blood Calcium After Calcium and Parathyroid	Added Ultraviolet Ray Exposure	Therapeutic Improvement
1. Atopic rhinitis	9.7	10.1	10.0	None
2. Atopic rhinitis	11.6	10.1	11.1	Mild
3. Atopic rhinitis	10.4	10.4	10.2	None
4. Atopic rhinitis	10.0	10.2	10.4	None
5. Asthma	9.1	9.2	9.0	None
6. Asthma	10.8	9.7	10.2	None
7. Asthma	11.3	11.0	11.4	Good for 2 months
8. Asthma	10.9	10.6	10.4	None
9. Asthma	9.7	10.3	9.9	None
10. Hay-fever	9.9	10.3	10.5	None

the figure, when the subject has a normal blood calcium. At least such an influence could not be demonstrated by the examination of the blood for calcium. It is true that as Kahn and Roe [11] have demonstrated, the blood calcium does increase a short time following the administration of calcium lactate, but, like the blood sugar curve, such a rise is not permanent but only transitory. The use of thyroid has been suggested on the assumption [12] that so many of these patients have a low basal metabolic

8. Sonnenschein, Robert; and Pearlman, S. J.: Correspondence, J. A. M. A. **84**:534 (Feb. 14) 1925; Calcium and Parathyroid Glands in Relation to Hyperesthetic Rhinitis, J. A. M. A. **83**:1973 (Dec. 20) 1924.

9. Brown, G. T., and Hunter, O. B.: Calcium Deficiency in Asthma, Hay-Fever and Allied Conditions, Ann. Clin. Med. **4**:299, 1925.

10. Salvesen, H. A.; Hastings, A. B., and McIntosh, J. F.: J. Biol. Chem. **60**:327 (June) 1924.

11. Kahn, B. S., and Roe, J. H.: Calcium Absorption from the Intestinal Tract in Human Subjects: An Experimental Study, J. A. M. A. **86**: 1761 (June 5) 1926.

12. Novak, F. J.: Basal Metabolism in Hyperesthetic Rhinitis, Wisconsin M. J. **19**:534, 1921.

rate. A glance at table 3 leads one to suspect with Simpson [13] and Sonnenschein that the rate is usually either normal or slightly above normal. Only one patient in our series had a basal rate below normal. And this is as one would expect, for clinically these patients are invariably high strung and nervous.

The fact remains, however, that many atopic patients show signs of improvement especially after the intravenous administration of calcium. But in view of the evidence presented, it hardly seems likely that such therapy is on a specific basis. We believe, rather, that what influence is seen is the result of the depressing action which calcium has on all the tissues, especially on the nervous system, as well as because it has a tendency to lessen the permeability of tissue cells and in that way reduce transudation, which is such a common manifestation in the shock organ of atopic persons.[14]

TABLE 3.—*Basal Metabolic Rate in Atopy*

Number	Name	Diagnosis	Rate	Number	Name	Diagnosis	Rate
1	.....	.....	....	10	J. B.	Urticaria.....	+38
2	J. G.	Atopic rhinitis	+ 3	11	A. G.	Urticaria.....	+14
3	M. M.	Atopic rhinitis	+ 7	12	E. T.	Urticaria.....	+29
4	L. S.	Asthma.....	+12	13	E. M.	Urticaria.....	+10
5	L. R.	Asthma.....	+11	14	D. J. McM.	Urticaria.....	+ 1
6	E. F.	Asthma.....	−10	15	G. C.	Urticaria.....	+ 2
7	M. N.	Asthma.....	+14	16	A. F.	Migraine.....	+ 6
8	A. L.	Asthma.....	+23	17	M. M.	Angioneurotic edema.....	+ 5
9	E. Z.	Asthma.....	+ 1				

ATOPY AND GASTRIC ANALYSIS

For some time past, we have been impressed with the occasional improvement that is seen in urticaria following the administration of dilute hydrochloric acid. This interest was stimulated further by Beckman [15] who states in a recent report that he has obtained 100 per cent symptomatic cure in a series of seventeen cases of hay-fever by the oral administration of nitrohydrochloric acid. Reference to the older literature reveals that Bishop,[16] Lockard [17] and others have used this drug, and even lemonade, for similar purposes with a great deal of success. It was thought worth while, therefore, to determine whether there is any

13. Simpson, W. L.: Basal Metabolism in Hyperesthetic Rhinitis and Bronchial Asthma, Laryngoscope **32**:768, 1922.

14. New and Nonofficial Remedies, Chicago, A. M. A. Press, 1927, p. 109. Editorial: Administration of Calcium Salts, J. A. M. A. **89**:968 (Sept. 17) 1927.

15. Beckman, Harry: Nitrohydrochloric Acid in the Treatment of Hay-Fever, Am. J. M. Sc. **174**:525, 1927.

16. Bishop, S. S.: Diseases of Ear, Nose and Throat, Philadelphia, F. A. Davis Company, 1897.

17. Lockard, L. B.: The Treatment of Hay-Fever, Boston M. & S. J. **148**:59, 1903.

change in the gastric secretion of atopic patients which may suggest the basis for such therapeutic results. This was undertaken not unmindful of the difficulty of interpretation of results of gastric analysis from the standpoint of acidity. For the purpose of control, gastric analysis employing the Rehfuss technic was performed on sixty-five apparently healthy medical students. Of the entire class, there were three cases of achlorhydria, and two of these gave a history of atopy, one of hay-fever and the other of urticaria. Fifty atopic patients were then studied from this standpoint (table 4). The gastric contents were examined in a few cases by the Ewald method and in the rest by the Rehfuss method. Twenty-eight of the fifty patients had asthma; six, urticaria; twelve, hay-fever; two, atopic rhinitis, and one, angioneurotic edema and one, migraine. Nineteen, or 36 per cent, of the entire series, had a complete lack of hydrochloric acid, and four, or 8 per cent, showed complete achylia. Sixteen, or 32 per cent, revealed hypo-acidity; fourteen, or 28 per cent,

TABLE 4.—*Gastric Analysis in Atopy*

	Number of Cases Studied	Achylia	Achlorhydria	Hypo-acidity*	Normal†	Hyper-acidity‡
Asthma	28	2	10	12	12	0
Hay-fever	12	0	4	4	4	1
Urticaria	6	2	3	1	3	0
Atopic rhinitis	2	0	1	0	1	0
Angioneurotic edema	1	0	1	0	0	0
Migraine	1	0	0	0	0	1
Total series	50	4	19	17	20	2

* Hypo-acidity—any value under 10.
† Normal—any value up to 70.
‡ Hyper-acidity—any value above 70.

were normal and three had hyperacidity. Further examination of table 4 shows that the same is true of the individual conditions considered. This is highly interesting, for a careful search of the literature fails to make mention of such an observation or to explain it. Particularly is it significant because atopic conditions are thought to be associated chiefly with vagotonia, which is closely related to hyperacidity instead of hypo-acidity. These observations are at least suggestive, for it appears that while achlorhydria is not constant in atopy, it is certainly encountered in this condition much more frequently than in normal persons.

CONCLUSIONS

1. The normal blood calcium established in our series is 10.43 mg. per hundred cubic centimeters of blood.

2. A calcium deficiency cannot be shown to exist in atopic conditions.

3. Calcium therapy does not seem to produce a permanent increase in the blood calcium of atopically sensitive patients.

4. The basal metabolic rate is not low in these patients; hence, thyroid is contraindicated.

Gastric analysis suggests the somewhat frequent presence of an achlorhydria and hypo-acidity in a large percentage of atopic conditions; hence, the advisability of investigating further the effect of acid therapy.

1004 May Building.

A COMPARISON OF THE EFFECTS OF GENERAL DIETS AND OF STANDARDIZED DIETS ON TOLERANCE FOR DEXTROSE *

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In a previous study [1] it has been shown that it is possible to change materially a subject's tolerance for dextrose by the use of different types of diet. The different types of diet used to demonstrate this effect were: one consisting strictly of protein, one of fats, one of carbohydrates; in another group food was not taken. The subjects of this experiment were normal, healthy, male medical students. They were instructed to follow certain of the dietary outlines mentioned for two days, and on the third morning, on a fasting stomach, a dextrose tolerance test was run on each subject with the use of 1.75 Gm. of dextrose per kilogram of body weight. Briefly, the results were: the subjects who had consumed only carbohydrates possessed a markedly increased tolerance for dextrose; those who had eaten only proteins showed a mild inability to remove the dextrose from the blood stream; whereas the subjects who had taken fats or who had starved for a period of two days showed a marked decrease in their tolerance for sugar as judged by the clinical dextrose tolerance test.

It was suggested that possibly some of the variability seen in the dextrose tolerance curves in normal persons might be eliminated if a standardized diet was followed for a few days prior to the performance of the dextrose tolerance test. It was further suggested that some of the dextrose tolerance curves believed to be characteristic of certain pathologic disorders might, in reality, be the effect of the diets taken because of the pathologic conditions.

I have attempted to study the effect of a standardized diet on the clinical dextrose tolerance test. Healthy, young, male medical students were again used for the purpose of studying this effect. Their basal requirement in calories was determined, and 25 per cent was added for the moderate activity of these students. The total calories were divided as follows: 0.66 Gm. of protein per kilogram of body weight, 2.5 Gm.

* From the Departments of Internal Medicine and Physiology, Baylor University, College of Medicine.

1. Sweeney, J. Shirley: Dietary Factors That Influence the Dextrose Tolerance Test, Arch. Int. Med. **40**:818 (Dec.) 1927.

of carbohydrate per kilogram and the remaining calories were supplied in fats. This gave what appeared to be an average balanced tray. The students all felt that it was approximately what they had been eating, except perhaps for a slight deficiency in bread. The students continued on these diets for two days. On the third morning, without breakfast, they reported for a determination of the tolerance for dextrose.

TABLE 1.—*Results of Dextrose Tolerance Tests on Students Who Had Been on Standardized Diet for Two Days*

	Blood Sugar Milligram per Hundred Cubic Centimeters			
Subject	Fasting	Thirty Minutes	Sixty Minutes	One-Hundred and Twenty Minutes
1	77	187	133	146
2	80	160	182	100
3	80	200	182	133
4	93	133	87	56
5	87	129	125	53
6	76	121	93	52
7	103	215	133	105
8	76	243	108	78
9	60	145	78	61
10	63	170	143	108
11	71	167	158	158
12	54	121	143	120
13	53	91	59	44
14	71	182	167	138
15	70	153	108	75
16	78	111	125	91
17	77	121	137	103
18	93	98	114	111
19	56	91	82	66
Total average	75	149	145	95
Average of first thirteen subjects	75	160	125	93

One and seventy-five hundredths grams of dextrose was given per kilogram of body weight. Specimens of blood were taken from the vein before the administration of the dextrose and at thirty, sixty and one hundred and twenty minutes following the administration. I made all sugar determinations by the Folin-Wu method.

Twenty-one students were treated in this manner. For controlled studies, an attempt was made to use the same students at a later date and to repeat the tolerance tests without their having followed any particular diet. In this way, it was possible to see the type of curve that a person would have following a standard diet and following an ordinary general diet. It was impossible in every instance to repeat the tolerance tests on the same students. There were, however, thirteen

cases in which I was able to study the effect of controlled and uncontrolled dieting on the tolerance for dextrose in the same students. Although this number is small, the variation noted is so insignificant that it is felt a larger group of observations would not change the results.

In table 1 the results of the dextrose tolerance test are listed for nineteen students who had taken a standardized diet for a period of two days prior to the test. In table 2, the results of the test for eighteen students are given; some of the students are the same as in table 1,

TABLE 2.—*Results of Dextrose Tolerance Tests on Students Who Had Been on General or Uncontrolled Diets*

	Blood Sugar Milligram per Hundred Cubic Centimeters			
Subject	Fasting	Thirty Minutes	Sixty Minutes	One-Hundred and Twenty Minutes
1	57	143	125	75
2	59	129	182	108
3	56	129	93	64
4	78	111	91	100
5	83	118	74	56
6	74	112	89	59
7	67	100	108	103
8	63	143	89	50
9	77	129	69	61
10	69	234	107	53
11	63	167	174	133
12	100	133	190	107
13	59	151	68	75
14	83	174	250	111
15	74	133	85	89
16	63	89	90	49
17	71	91	105	82
18	77	100	105	59
Total average	71	133	116	80
Average of first thirteen subjects	70	138	112	80

whose tests were run following undirected eating habits. The figures on the first thirteen students in each table are the results of the dextrose tolerance tests on the same students following the eating of a standardized diet and without any dietary direction. The figures as presented are shown graphically in charts 1 and 2, as composite curves. In chart 1, the composite curves of the students are compared, with and without the effect of standardized dieting. It should be remembered that not all of these students are the same in both cases. In chart 2, the composite curves for thirteen students are shown, identical in both groups, following the taking of standardized diets and following normal dietary habits.

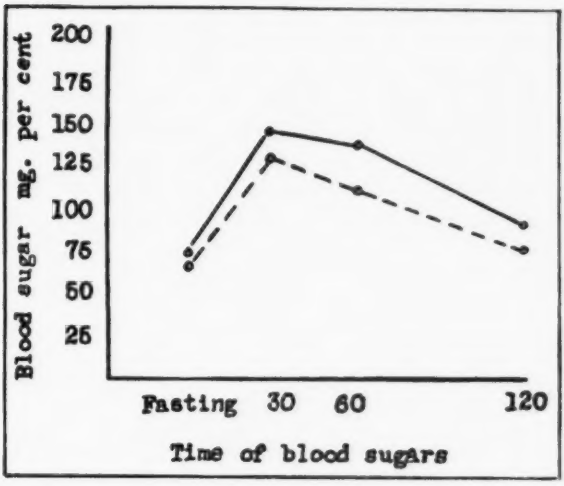

Chart 1.—Average or composite curves of the students with and without the effect of standardized dieting. The dash line represents those who had taken a general diet.

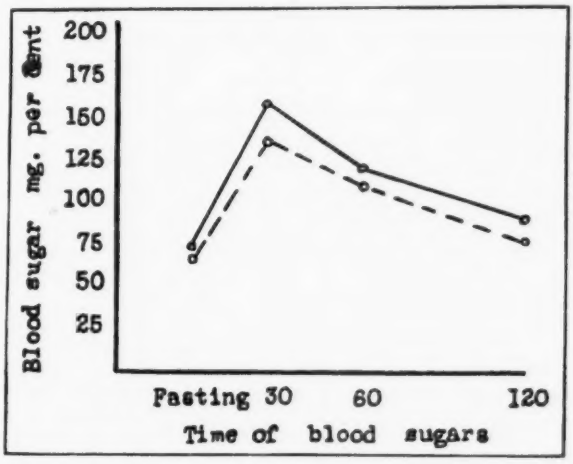

Chart 2.—Average or composite curves of thirteen students, identical in both groups, with and without the effect of an antecedent standardized diet. The dash line represents those who had taken a general diet.

COMMENT

It is not difficult to see that the effect of the preceding diets, as used in this study, is only slight. In chart 1, it will be noted that the curves, although similar in form, might differ significantly quantitatively. This is probably due to the fact that the results for different students were included in these two curves. There is something in a person's metabolic mechanism, regardless of how many variables might be held constant, that causes him to respond in a certain way to dextrose. It will be noted in chart 2, in which the same students are represented in each curve, that the difference is negligible as far as the curves per se are concerned. The slight quantitative difference that exists between the observations is probably to be accounted for by the fact that those who were on the general diets consumed slightly more carbohydrates, which, it is known, will increase a person's tolerance materially, other things being equal. There are no differences noted great enough to allow one to say that an ordinary well mixed general diet in any way distorts one's tolerance to sugars. The suggestion previously made, as alluded to in the beginning, that a standardized diet would perhaps make less variable dextrose tolerance curves, is not borne out. The individual curves included in this study are not plotted because of the requirement of space. Inspection of them, however, or reference to the tables, convinces one that the curves for the thirteen students, on standardized diets, varied just as much as they did for those on an uncontrolled diet.

CONCLUSIONS

Healthy, young, medical students were studied in respect to their tolerance for dextrose, following antecedent standardized or controlled dieting and following uncontrolled dieting. It was found that antecedent standardized diets practically did not have any influence on the variability of the curve for dextrose tolerance as compared to the curves made after the subject had been on an ordinary general diet. There was a slight quantitative difference in the composite curves, which was explained on the basis of the difference in the composition of the diets.

Medical Arts Building.

THE UREA TOLERANCE TEST

AN INDEX OF RENAL FUNCTION *

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This investigation was undertaken to determine whether the changes in blood urea concentration following the ingestion of urea could be utilized in the estimation of kidney function. Certain properties of urea, which are generally recognized, make this substance suitable for such purposes.

1. Urea is extremely soluble. It is rapidly absorbed unchanged from the gastro-intestinal tract.[1]

2. It is distributed in uniform concentration in practically all the tissues of the body.[2]

3. It is nontoxic even in large doses.[3]

4. Urea is a normal metabolite and a natural stimulus to the kidneys in direct proportion to its concentration in the blood.[4]

5. The determination of blood urea nitrogen is simple and accurate.

6. Under normal conditions, urea is excreted chiefly by the kidneys; elimination through the intestine and skin being negligible.[1]

There is some evidence indicating that urea is more than an inert excretory product of nitrogen metabolism. The addition of urea to perfusion fluids produces stimulation of cardiac contraction. It is said to possess a minor rôle in the acid base equilibrium.[1] Even the simple carbamide structure of the urea molecule is questionable since an

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1. Fearon, W. R.: The Biochemistry of Urea, Physiol. Rev. **6**:399 (July) 1926.

2. Marshal and Davis: The Urea Distribution and Elimination of the Body, J. Biol. Chem. **18**:53, 1914.

3. Volhard, F.: Die doppelseitigen hematogenen Nierenkrankheiten, Berlin, 1918. Feichenfeld, J.: Ueber Harnstoffe als Diureticum, Therap. d. Gegenw. **59**:273, 1918. Strauss, H.: Ueber Harnstoff als Diureticum, Berl. klin. Wchnschr. **58**:375, 1921. King, S. E.: The Diuretic Action of Urea and High Protein Diets, M. Clin. N. Amer. **10**:963 (Jan.) 1927.

4. MacLean, H.: Modern Methods in the Diagnosis and Treatment of Renal Disease, London, 1921. Ambard, L., and Weill, A.: Les lois numeriques de la secretion renal de l'uree et du chlorine de sodium, J. de physiol. expér., 1912, vol. 14. Austin, J. H.; Stillman, E., and Van Slyke, D. D.: The Factors Influencing the Excretion of Urea, J. Biol. Chem. **46**:91, 1921. McLean, F. C.: The Numerical Laws Governing the Rate of Excretion of Urea and Chlorides in Man, J. Exper. Med. **22**:212, 1915. Addis, T., and Drury, D. R.: The Effects of Changes in Blood Urea Concentration on the Rate of Urea Excretion, J. Biol. Chem. **55**:105, 1923.

isomeric cycle form has been shown to exist.[4] the original conception prevails that urea is largely a waste product excreted almost entirely by the kidneys. The small fraction of ingested urea diverted to other channels is probably more than compensated for by the endogenous quota formed by tissue catabolism.

Speculations regarding a protein sparing function of ingested urea are apparently unsubstantiated by the existing evidence for the irreversibility of urea formation in the body.[4]

The importance of these premises is apparent, since the validity of all kidney function tests in which urea is employed, is dependent on their verity.

The idea of estimating renal function, by the rapidity of elimination from the blood of previously ingested urea, is not original. A survey of the literature, however, yielded only two references. One of these is by Archer and Robb,[5] and the other is a recent one by Rhodebeck,[6] which appeared in the bulletin of a local hospital.

Rhodebeck failed to obtain results of any practical value. The factor responsible for his observations is discussed in detail subsequently in this paper. At present, it is sufficient to point out that the excessive fluid intake prescribed in his cases produced a diuresis which entailed a secondary increase in the rate of urea elimination from the blood.

Archer and Robb apparently were the first to attempt the estimation of kidney function by a blood urea curve following the ingestion of urea. Unfortunately, the method which they employed was entirely inadequate, since the measurable factor was dominated by incidental variations in blood urea. The bizarre urea values presented by them cast doubt on the accuracy of their micromethod; there was no limitation of fluid intake in their subjects; the period allotted to their test was too short (two hours) to allow the blood urea to return to the control level in normal persons and to permit of proper interpretation of the effects of the ingestion of urea. By following the curve to the rest level as has been done in this series the measurable element is considerably increased and consequently overshadows any chance variations.

CONTROL OBSERVATIONS

The Normal Blood Curve Following Varying Doses of Urea.—The results in a small group of normal subjects are indicated in chart 1, which may be summarized as follows:

1. Large doses of urea, 20 Gm. or over, produced an elevation in the blood urea nitrogen reaching a maximum in from two to four

5. Archer, H. E., and Robb, G. D.: The Tolerance of the Body for Urea in Health and Disease, Quart. J. Med. **18**:274, 1925.

6. Rhodebeck, E. J.: Study in Urea Feeding: Preliminary Report, Fifth Avenue Hosp. Clinics, New York, 1927.

hours, depending on the dose. The maximal level obtained was proportional to the amount of urea ingested.

2. The time required for the blood urea to return to the control level similarly appeared dependent on the amount of urea ingested. Because of the prolonged elevations occurring with large doses of urea, the actual time required for the blood urea nitrogen to return to the rest level was not determined, since it appeared probable that these

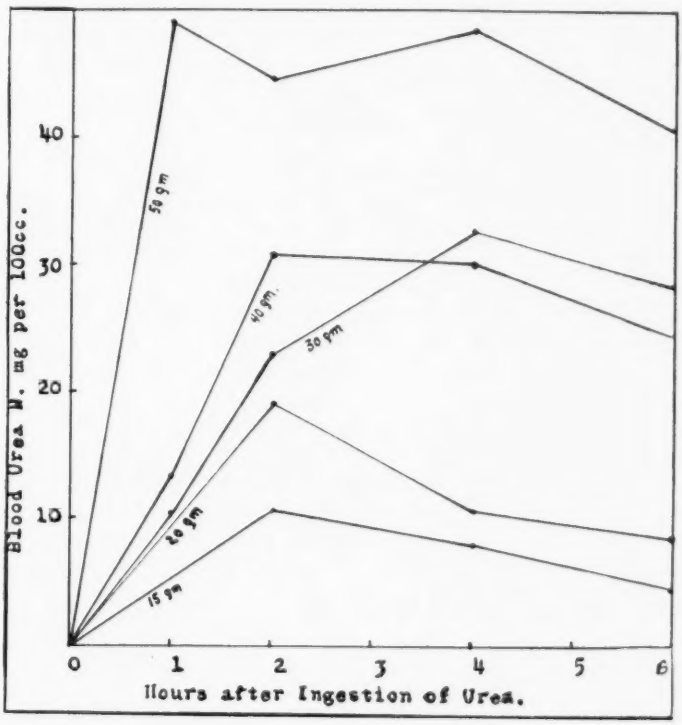

Chart 1.—Blood urea curves following graduated doses of urea. The abscissas represent the elevation above rest level.

would persist for longer periods than could reasonably be allotted to the procedure.

The Normal Blood Curve Following 1 Gm. of Urea per 10 Pounds of Body Weight.—A composite curve using 1 Gm. of urea per 10 pounds of body weight, or 15 Gm. of urea for an average person weighing 150 pounds (68 Kg.), is shown in chart 2. Sixty-five determinations of the blood urea nitrogen in twenty-seven normal cases are plotted

at various intervals following the ingestion of urea. These results may be summarized as follows:

1. Under the conditions of our observations, the ingestion of this amount of urea by normal subjects produced a sharp elevation in the blood occurring between the first and second hour and followed by a gradual fall to the control level within fourteen hours.

2. The maximal elevation usually occurred about the first hour, its extent varying, however, in individual cases. In some cases, elevations as high as 14 or 15 mg. of urea nitrogen above the rest level were obtained.

3. The blood urea level at the second hour was subject to less variation. The average elevation above the rest level was 10.1 mg. of blood urea nitrogen.

This is of considerable interest, since it checks closely with the results of Marshal and Davis [2] who calculated that 15 Gm. of urea at equilibrium produced a rise of about 10 mg. of blood urea nitrogen in a person weighing 150 pounds (70 Kg.). Rabinowitch,[7] incident to a study of the MacLean test, reported an average elevation of 9.5 mg. of blood urea nitrogen, two hours following the ingestion of 15 Gm. of urea in 200 normal subjects.

The explanation of the fluctuations occurring during the early period of absorption is indicated by the work of Marshal and Davis. Following the intravenous injection of urea into animals, they discovered that the immediate elevation in the blood urea was followed by a rapid fall, which they ascribed to diffusion into the tissues. Eighteen minutes following the injection, equilibrium between blood and tissue had not yet been reached. It is obvious that an even longer period would be necessary to establish equilibrium following oral administration.

In view of the relative constancy in the concentration of blood urea two hours following ingestion, it was concluded that this represented the equilibrium level and was selected as a critical point for comparative observations. At this time, the blood urea concentration is roughly dependent on the body weight, i. e., the amount of tissue available for the diffusion of urea.

Urea diffuses readily into all the tissues excepting bone, fat, cartilage and dermal structures. According to Marshal and Davis,[2] over 90 per cent of all tissues contain urea. Similarly it diffuses into exudates and edema fluid.[8] At equilibrium, the concentration of urea in the blood and tissues is identical, with the exception of the tissues mentioned and

7. Rabinowitch, I. M.: The Urea Concentration Test, Arch. Int. Med. **28**:827 (Dec.) 1921.

8. Dennis, W., and Minot, A. S.: The Nonprotein Constituents of Edema Fluids, Arch. Int. Med. **23**:879 (Dec.) 1917.

the kidneys, in which the concentration of urea is much higher than in the blood. This does not necessarily indicate storage of urea, since in the chemical analysis of kidney tissue contamination with urine is unavoidable.

Excretion of urea by the kidneys and its distribution throughout the tissues, occurring simultaneously, accounts for the sharp fall which frequently occurs in the blood concentration, from the early maximum

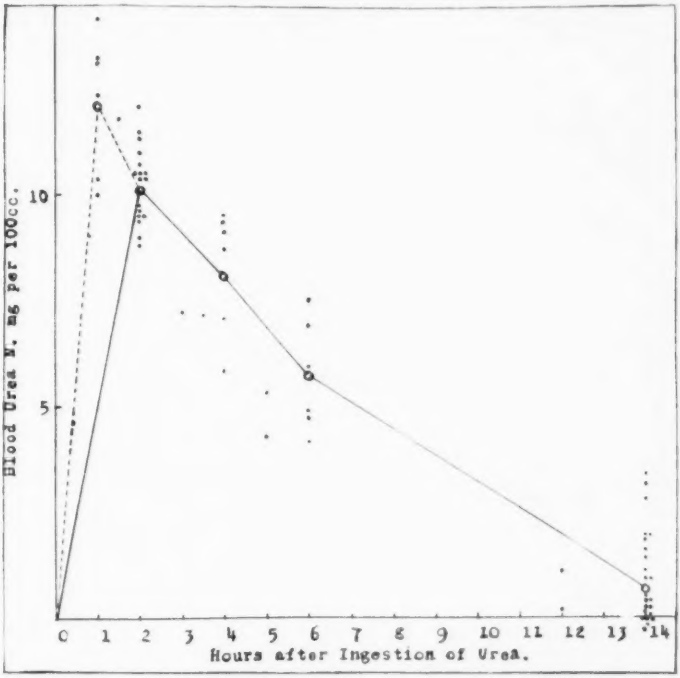

Chart 2.—The normal blood urea curve following the ingestion of 1 Gm. of urea per 10 pounds of body weight. A composite curve of sixty-five estimations at varying periods after urea ingestion in twenty-seven normal subjects. The abscissas represent the elevation above rest level. The circles indicate average values.

level to the point of equilibrium. The rate of urea excretion is greatest when the concentration of urea in the blood is highest. From 3 to 4 Gm. of urea may be excreted in the urine in the first two hours after ingestion. The importance of this factor is indicated by the high levels of blood urea occurring at the point of equilibrium in cases with impaired renal function.

In order to produce a similar rise of 10 mg. of blood urea nitrogen two hours after the ingestion of the urea in persons of varying weight, corrections are necessary on the basis of 1 Gm. of urea for every 10 pounds deviation from 150 pounds. Thus, a person weighing 120 pounds (54.4 Kg.) would require a test dose of 12 Gm. of urea, and one weighing 170 pounds (77.1 Kg.) would require 17 Gm. Similar adjustments are necessary for edema fluids and effusions. The reason for these corrections is the necessity of producing elevations of blood urea as nearly identical as possible. To obtain comparable results, the response of the kidneys to an identical stimulus must be studied, this being the elevation in the blood urea nitrogen.

The rate of absorption from the gastro-intestinal tract undoubtedly modifies these conclusions. It seemed doubtful whether the elimination of this factor through the intravenous administration of urea would be worth the technical difficulties introduced, and from previous considerations, it appeared improbable that it could be entirely eliminated.

TABLE 1.—*Blood Urea Variations Over a Fourteen-Hour Fasting Period*

	Mg. of Blood Urea Nitrogen per 100 Cc.			
Case	Control	After 14 Hours	Variation	Kidney Function
H.	18.1	20.2	+2.1	Normal
B.	21.2	18.9	−2.3	Normal
M.	14.7	15.2	+0.5	Normal
M.	7.0	6.7	−0.3	Normal
R.	42.8	40.8	−2.0	Severely impaired

4. In all normal cases, the blood urea nitrogen had fallen within 2 mg. of the rest level at the end of fourteen hours. In many cases, the return to rest level occurred even after twelve hours, but since this was not uniformly true, fourteen hours was chosen as the period for observation. Objections to the length of this period, representing one of starvation, were overcome to a great extent by the administration of urea in the evening carrying the test through the sleeping hours.

A few control observations were made to study the normal variation in blood urea occurring over a fourteen-hour fast period. In four normal cases and in one of severe nephritis, the variation between the values at the beginning and at the end of this period did not exceed 2.3 mg. of urea nitrogen (table 1).

These results check closely with the observations of MacKay and MacKay,[9] who found the hourly variation in blood urea nitrogen did not exceed 3 mg. in six normal subjects, who were studied over long periods under ordinary conditions. It would be expected that the variations in blood urea would be diminished under the conditions of our

9. MacKay, E., and MacKay, L. L.: The Concentration of Urea in the Blood of Normal Individuals, J. Clin. Investigation **4**:295, 1927.

observations, as the subjects stayed in bed and did not receive any food during the period studied.

One gram of urea for each 10 pounds of body weight or 15 Gm. for an average adult weighing 150 pounds was chosen as the standard dose for the following reasons:

1. The period required for the blood urea to return to the control level is considerably shorter than that with larger doses.

2. Smaller amounts of urea produce an insufficient rise in the blood to be of value in plotting a curve.

3. This is the dose employed in the MacLean urea concentration test, which could be performed simultaneously, if desired.

4. Although this amount of urea is ingested without difficulty, larger doses may occasionally produce disturbances of the gastro-intestinal tract.

Calculation of the dose of urea in grams and the body weight in pounds may provoke criticism. The utility of this step is obvious, since by so doing, decimal points and fractions are eliminated from the calculations.

The Influence of the Volume of Urine on the Excretion of Urea.—In the routine adopted in this test, about 500 cc. of fluids was permitted, the purpose being to obtain moderate volumes of urine for the fourteen-hour period.

The influence of the volume of urine on the rate of excretion of urea has been the subject of considerable investigation since the work of Ambard. Austin, Stillman and Van Slyke [10] concluded that the rate of excretion of urea was proportional to the square root of the volume of urine within certain limits. This mathematical relationship has not been entirely confirmed. Addis [11] came to the conclusion that a relationship between the volume of urine and the rate of excretion of urea existed only with oliguria, and that with increasing volumes of urea the rate of excretion of urea was augmented only temporarily. Within certain limits, however, it is agreed that the volume of urine does influence the rate of excretion of urea.

This feature was illustrated by certain observations in a normal subject, who did not take the full amount of fluid allotted and perspired considerably during the course of the test. The volume of urine for the fourteen hour period in this case was only 150 cc. A persistent elevation amounting to 3.6 mg. of blood urea nitrogen was present at

10. Austin, Stillman and Van Slyke (footnote 4, third reference).

11. Addis, T., and Watanabe, J.: The Effect of Changing the Volume of Urine on the Excretion of Urea, J. Biol. Chem. **29**:399, 1917. Addis, T., and Drury, D. R.: The Effect of Changes in Urine Volume on the Rate of Urea Excretion, J. Biol. Chem. **55**:639, 1923.

the end of the test. Repetition of the procedure with provisions for an adequate volume of urine yielded a normal curve (case A. F., table 2). The excretion of 15 Gm. of urea in 150 cc. of urine is apparently beyond the capacity of the normal kidney. This would necessitate concentration of urea in the urine of 10 per cent, which Ambard,[12] Addis [13] and others [14] have shown does not occur even under the most unusual conditions.

Case B. C., table 2, illustrates the effect of increasing the fluid intake in a patient with slightly impaired kidney function. A slight retention in the blood curve initially present disappeared when the volume of urine was increased by the forced ingestion of fluid. In case M. N. (table 2) with kidney function severely impaired, increasing the urine volume from 700 to 1,100 cc. markedly diminished the blood urea retention. In patients with impaired kidney function, polyuria

TABLE 2.—*Influence of the Volume of Urine on the Blood Urea Curve*

	Urea Test					
	Mg. of Blood Urea Nitrogen per Hundred Cubic Centimeters					
		After Urea				
Case	Control	2 Hrs.	14 Hrs.	Retention	Urine Volume	Clinical Diagnosis
A. F.	13.0	23.9	16.6	3.6	150	Normal
	18.0	....	18.6	0.6	650*	
B. C.	16.1	29.5	18.6	2.5	500	Nephritis of pregnancy
	17.1	31.0	17.5	0.4	1025*	
M. N.	20.4	36.0	32.8	12.4	700	Chronic nephritis
	26.9	46.4	34.0	7.1	1100	

* Increased fluid intake.

appears to exist as a compensatory mechanism to permit the elimination of urea and other waste products.

The proper evaluation of the changes in the blood urea following the ingestion of urea demands that the volume of urine must be within certain limits. The optimal volume in this kidney function test is about 500 cc. Under such conditions, considerable work is required from the kidney which is forced to excrete urea in concentrations over 3 per cent to prevent retention in the blood. Increased fluid intake or even neglect in restricting fluids will normally result in polyuria, owing to the intrinsic diuretic action of the ingested urea. Under such con-

12. Ambard (footnote 4, second reference).

13. Addis, T., and Foster, M. G.: The Concentrating Capacity of the Kidneys, Arch. Int. Med. **34**:462 (Oct.) 1924.

14. Adolph, E. F.: Excretion of Water by the Kidneys, Am. J. Physiol. **65**:419, 1923. Volhard (footnote 3, second reference). Ambard (footnote 4, third reference).

ditions, the capacity of the kidneys to perform work, i. e., to concentrate urea in the urine, cannot be properly studied.

Some recent work by Rhodebeck [6] published during the course of this investigation illustrates the erroneous results that may be obtained if this factor is neglected. He reported similar blood curves following the ingestion of urea by normal persons and in persons with nephritis. His results are readily explained by the conditions of his experiments, in which excessive fluid intake produced a marked polyuria. Under such conditions, urea is eliminated with equal facility by normal and by some impaired kidneys.

THE METHOD OF STUDY

Although slight changes were at first necessary, the following method was subsequently adopted for purposes of uniformity and simplicity.

1. Supper was not usually given on the evening of the test. A meal low in protein content, consisting of fruit or a vegetable salad, taken several hours before the test did not appear to affect the results materially. Moderate restriction of fluid, however, was found to be essential.

2. In the evening at the beginning of the test, the patient voided, and the specimen was discarded. He was then instructed to collect all urine until the test was completed.

3. At this time, an oxalated specimen of blood was collected.

4. The patient was then given 1 Gm. of urea for each 10 pounds of body weight, i. e., 15 Gm. for an adult weighing 150 pounds. Crystalline urea was dissolved in about 8 ounces of well sweetened lemonade. It was found that urea is best taken in such a vehicle. Tea and coffee were avoided because of their diuretic action. It is necessarily essential to insure the complete ingestion of the urea. If vomiting occurred, the test was abandoned. This, however, took place only once in our series.

5. Two hours following the ingestion of urea, a second specimen of blood was collected. When inconvenient, this specimen was omitted without materially affecting the interpretation of the test.

6. To insure both ease of supervision and to diminish metabolism, the patient stayed in bed during the entire period of observation. Since this occurred at night, it was not found irksome.

7. The following morning, fourteen hours after the ingestion of urea, the last specimen of blood was taken. At this time, the volume of urine was measured, thus completing the test.

This method was found simple in execution and interfered little with hospital routine or the comfort of the patient. The omission of

the second specimen of blood permitted the use of this test in some cases in the outpatient department.

The method used for the estimation of blood urea nitrogen is the one described by Myers.[15]

INTERPRETATION

1. It was found that for all practical purposes, the concentration of blood urea at the second hour yielded relatively minor information, except that it indicated adequate absorption of urea. Although in patients with severely impaired kidney function the elevation was usually of greater extent than in the normal person, this varied even in the same person.

2. The elevation of blood urea nitrogen persisting above the rest level fourteen hours after the ingestion of urea was employed as the basis of interpretation of this test.

Variations up to 2 mg. of blood urea nitrogen above the control level were considered within normal limits, providing the output of urine was not excessive.

3. Under the conditions of this procedure in which excessive intake of fluid was prohibited, a urine volume of over 750 cc. for the fourteen-hour period was considered abnormal.

CLINICAL APPLICATION

It is generally recognized that for most practical purposes the fasting blood urea is a good index of kidney function. MacKay and MacKay [16] have recently shown that an increase in fasting blood urea does not occur until over 50 per cent of the kidney structure has been destroyed. When kidney function is grossly impaired as indicated by the functional tests in common use, any new modification or procedure is superfluous. It is only in the larger group of cases in which insidious pathologic changes of the kidneys are present, which are not indicated by casual examination, that a new test may be of value.

There are too many unknown variable factors influencing the function of the kidneys for the results of any test to be strictly quantitative in character. It is not claimed that the degree of retention of urea strictly parallels the extent of pathologic change in the kidneys. That function and structure are not always parallel is generally recognized. The purpose has been to determine if minor degrees of impaired kidney function could be detected which under ordinary condi-

15. Myers, V. C.: Practical Chemical Analysis of the Blood, St. Louis, C. V. Mosby Company, 1924, p. 45.

16. MacKay, E., and MacKay, L. L.: The Relation Between the Blood Urea Concentration and the Amount of Functioning Renal Tissue, J. Clin. Investigation **4**:127, 1927.

tions escape observation. By subjecting the kidney to the necessity of eliminating a relatively large amount of urea in a limited volume of urine, conditions of strain are produced to which only a perfectly functioning kidney can respond normally. It has been previously shown that if these conditions be made still more severe by further decreasing the urine volume even the normal kidney becomes inadequate to the task of complete elimination of the additional urea in the given time.

The material in this study was divided into three groups from the point of view of renal function: a normal group; one in which renal function was definitely impaired, and a third in which impairment

TABLE 3.—*Urea Test in Patients with Definitely Impaired Kidney Functions* *

		Urea Test					
		Mg. of Blood Urea Nitrogen per Hundred Cubic Centimeters					
	Blood† Urea Nitrogen Mg.		After Urea			Urine Volume in Cc.	
Case	100 Cc.	Control	2 Hrs.	14 Hrs.	Retention	14 Hrs.	Clinical Diagnosis
A. P.	20.4	20.4	38.7	36.3	15.9	640	Chronic diffuse nephritis
J. R.	55.6	47.1	63.4	61.0	13.9	700	Chronic nephritis
C. G.	18.0	18.6	31.8	27.9	9.3	400	Syphilis, amyloidosis
M. G.	11.7	11.5	27.4	20.7	9.2	800	Bilateral cystic kidney †
J. P.	32.5	25.9	35.0	31.2	5.3	1200	Pyelonephritis, chronic nephritis §
P. A.	38.4	26.0	39.4	35.1	9.1	900	Paralysis of bladder, tumor of the cord ‡
E. K.	34.8	20.1	34.4	27.6	7.5	1500	Calculus in renal pelvis, pernicious anemia ‡
J. L.	12.6	9.8	21.0	18.4	8.6	1050	Chronic nephritis
M. N.	19.7	20.4	36.0	32.8	12.4	700	Chronic nephritis §
J. M.	26.2	22.5	35.0	29.3	6.8	1120	Chronic nephritis
A. D.	75.0	70.2	82.6	85.0	14.8	400	Chronic nephritis, uremia §
I. W.	54.8	83.3	94.6	99.3	16.0	200	Chronic nephritis, uremia
L. D.	18.4	17.9	31.8	24.0	6.1	1100	Cerebrospinal syphilis, paralysis of bladder ‡
A. F.	19.4	25.0	44.0	32.1	7.1	700	Pyelonephritis
J. B.	14.2	12.3	....	23.6	11.6	400	Acute nephritis
F. B.	12.0	10.7	22.2	15.7	5.0	480	Pernicious anemia

* Protein, casts or cells were present in the urine in all cases. The two-hour and phenolsulphonphthalein test were impaired in all cases.
† On admission.
‡ Confirmed by exploratory operation.
§ Confirmed by tissue examination.

of renal function was suspected. The clinical observations, the routine examination of the urine, the fasting blood urea, uric acid and creatinine, the two-hour test, the phenolsulphonphthalein test and occasionally the MacLean test were used as the basis for classification. The results in the normal group have already been discussed.

The Blood Urea Curve in Patients with Definitely Impaired Renal Function.—The changes occurring in the blood urea in a group of sixteen patients with definitely impaired renal function are indicated in table 3 and chart 3. These patients showed evidence of a pathologic processes in the kidneys, indicated by large amounts of urinary protein, red blood cells or casts. In half of this group, initially elevated blood urea, uric acid and creatinine values were present. Impairment of renal function was evident by the abnormal two-hour tests and the diminished

excretion of phenolsulphonphthalein. The initially low blood urea values in some were deceptive, since they were attributable to previous prolonged protein restriction. In several of this group, the diagnosis was subsequently confirmed by autopsy. The results may be summarized as follows:

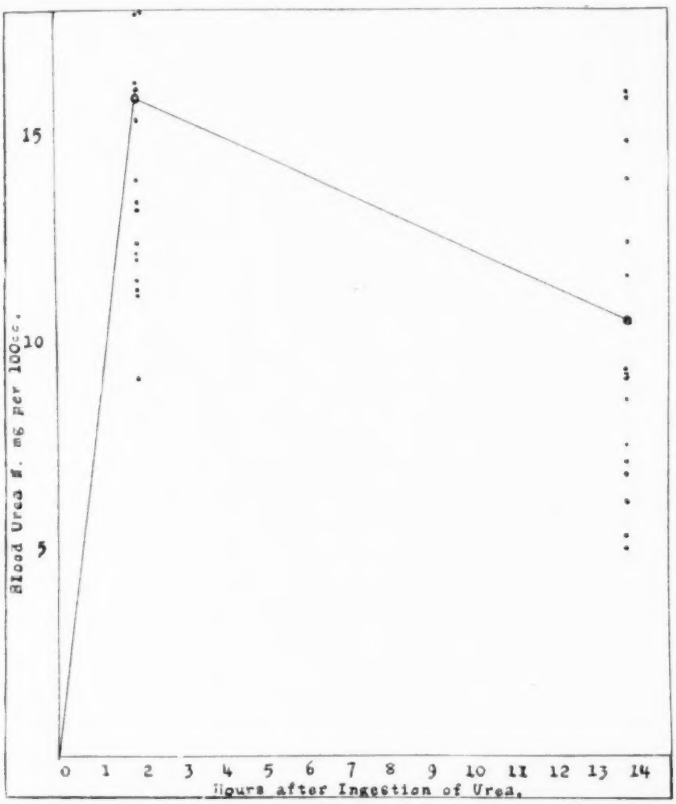

Chart 3.—The blood urea curve with kidney function severely impaired. A composite curve of sixteen cases with advanced renal functional impairment following the ingestion of 1 Gm. of urea per ten pounds of body weight. The abscissas represent the elevations above rest level. The circles indicate average values.

1. The blood urea, two hours following the ingestion of urea, was definitely higher than normal. The average elevation in terms of blood urea nitrogen was 15.9, contrasted with a normal average of 10.5. Individual elevations of over 18 mg. were obtained.

2. After fourteen hours, there remained an average residual elevation of 10.3 mg. above the control level. Consequently, on the average, less than one third of the urea given had disappeared from the blood.

3. In about half of these cases, a marked polyuria, over 750 cc., was present for the fourteen-hour period in addition to the abnormal blood curve.

4. In two cases of uremia, the blood urea continued to rise during the fourteen hours. Apparently, the kidneys were unable to eliminate not only the urea administered, but also that produced by catabolism of the tissues, which is known to be increased in this condition.[17] Another interesting feature was the loss of the compensatory polyuria, which was replaced by a relative oliguria in these two cases.

It is to be noted that in none of these patients who were severely ill did the ingestion of urea produce any discomfort or influence their clinical condition in any way.

The Blood Urea Curve in Patients in Whom Impairment of Renal Function was Suspected.—A third group of patients were studied in whom pathologic processes seemed present in the kidneys, and some impairment of renal function was suspected but not confirmed by the usual methods. The basis for selection of these patients was the presence of moderate to large amounts of protein, casts and red blood cells in the urine, with normal contents of the blood and normal, two hour and phenolsulphonphthalein tests. Included in this group were some patients in whom it is clinically recognized that depression of renal function may occur. The group consisted of cases of acute glomerular nephritis, early cases of chronic diffuse nephritis, nephrosis, infections and obstructive lesions of the urinary tract, chronic passive congestion due to cardiac disease and some cases of jaundice and severe anemia. The results in this group indicated in table 4, are classified under their clinical subdivisions.

Primary Renal Disease: Moderate retention of urea above the rest level after fourteen hours occurred in cases of acute nephritis, chronic nephritis, primary contracted kidneys and various infections and obstructions of the urinary tract. Even in early renal disease, such as in mild subsiding glomerular nephritis, nephrosis and minor infections of the urinary tract, the retention of urea though less marked was unmistakable. In some of these cases, diminished function was also made evident by a marked fourteen-hour polyuria.

Cardiac Disease: In the well compensated stage of cardiac disease, a normal response was obtained, and this was true even in some cases with edema. The fixation of the two hour test at a high level which is usual in these cases is probably dependent on disturbed excretion of

17. Mosenthal, H. O.: Metabolism in Nephritis: Endocrinology and Metabolism, vol. 4, New York, D. Appleton & Company, 1922, p. 337.

salt and water, which at first does not influence the elimination of nitrogen as long as the volume of urine is adequate. In one case, that of A. S. (table 4), retention of urea was noted during the stage of marked chronic passive congestion; subsequently a normal reaction to the test occurred when the efficiency of the circulation had been restored. That this retention was probably due to an actual disturbance in renal

TABLE 4.—*Urea Test in Patients in Whom Impairment of Kidney Function was Suspected* *

		Urea Test					
		Mg. of Blood Urea Nitrogen per Hundred Cubic Centimeters					
	Blood† Urea Nitrogen Mg.		After Urea			Urine Volume in Cc.	
Case	100 Cc.	Control	2 Hrs.	14 Hrs.	Retention	14 Hrs.	Clinical Diagnosis
T. C.	15.6	22.2	34.3	30.5	8.3	500	Chronic nephritis
M. W.	17.9	12.8	27.4	21.4	8.6	650	Acute nephritis
L. K.	19.7	21.4	28.8	25.0	3.6	450	Chronic nephritis
M. H.	16.6	18.0	40.0	28.4	10.4	900	Chronic nephritis, hypertension
H. G.	12.4	15.3	29.4	22.8	7.5	400	Tuberculosis of kidney
L. M.	29.8	12.3	26.7	19.8	7.5	600	Chronic nephritis, cardiac decompensation
M. H.	10.5	10.5	....	15.3	4.8	620	Pyelonephritis
R. G.	11.6	11.1	22.6	17.5	6.4	520	Renal tuberculosis
G. K.	11.7	12.1	21.6	16.8	4.7	450	Cystitis and pyelitis
A. B.	20.8	20.2	32.6	22.4	2.2	1400	Large renal calculus
C. M.	9.9	10.2	19.7	11.8	1.6	800	Pyelitis
M. B.	11.9	10.5	20.0	13.5	3.0	500	Pulmonary Tuberculosis, nephrosis
M. P.	28.0	15.2	34.4	20.4	5.2	...	Renal calculus, multiple abscesses of kidney
I. M.	11.1	10.0	17.3	10.3	.3	1800	Hydronephrosis
H. E.	11.3	11.3	....	14.4	3.1	500	Albuminuria
B. C.	....	16.1	29.5	18.6	2.5	500	Toxemia of pregnancy, hypertension
D. F.	18.4	17.2	....	20.6	3.4	...	Jaundice, cholecystitis
E. D.	22.0	22.9	36.0	29.8	6.9	500	Jaundice, ascites, syphilis of liver
A. D.	9.7	13.6	21.6	17.0	3.4	1400	Jaundice, hepatitis, renal calculus
A. S.	15.2	14.0	31.6	21.4	7.4	700	Cardiac decompensation, with ascites and edema
A. S.	14.6	12.2	19.8	12.5	.3	680	Cardiac, same case; compensated, no edema
M. L.	16.6	13.4	26.0	17.3	3.9	400	Jaundice, cardiac decompensation
G. R.	12.4	21.0	....	24.4	3.4	400	Jaundice, cancer of the liver
M. T.	10.5	8.9	16.4	13.6	4.7	600	Jaundice, severe anemia
N. B.	18.4	9.1	21.0	14.5	5.4	600	Pernicious anemia
W. T.	12.8	9.7	20.6	13.3	3.6	380	Pernicious anemia
P. C.	10.0	13.5	....	15.4	1.9	...	Hypertension
A. S.	12.1	18.6	....	19.0	.4	550	Essential hypertension
B. Z.	12.2	11.0	....	11.0	0	650	Hypertension, previous nephrectomy
J. M.	18.0	15.9	....	16.4	.5	...	Essential hypertension, aortitis
J. W.	....	9.0	....	9.9	.9	450	Essential hypertension

* The two-hour and phenolsulphonphthalein tests were normal in all cases. Protein, cells or casts were present in the urine in all cases.

† On admission.

function dependent on disturbed circulation rather than to oliguria was evidenced by the adequate volume of urine, of 700 cc. originally present during the fourteen-hour period. It is obvious that urea retention can occur in cases of chronic passive congestion with marked oliguria or anuria.

Essential Hypertension: In the absence of nephritis or contracted kidney, neither abnormal retention of urea nor polyuria occurred in five cases studied.

Jaundice: The occurrence of renal irritation incident to prolonged jaundice is generally recognized.[18] Protein and casts are frequently present in the urine in this condition. In several cases of hepatic disease with jaundice and ascites, moderate retention of urea occurred (cases D. F., E. D., M. L., G. R. and M. T., table 4). It seems hazardous to conclude that this indicates purely renal impairment, since the influence of the liver on urea metabolism is still poorly understood.

Anemia: The occurrence of impaired kidney function in pernicious anemia was first pointed out by Mosenthal [19] in a study of the two hour test, and subsequently by Christian.[20] The frequency of a mild pathologic process of the kidneys in this condition has recently been emphasized by Stieglitz.[21] In four cases of pernicious anemia, moderate retention of urea occurred in the blood following the ingestion of urea. Retention did not occur in several cases of secondary anemia.

The miscellaneous character of the remaining cases does not permit of any further clinical classification.

COMMENT

The purpose of this investigation has been to ascertain whether renal function can accurately be gaged by the capacity of the kidney to excrete large amounts of urea under special conditions. This has been done by a study of the changes occurring in the concentration of blood urea following the ingestion of 15 Gm. of urea for an adult weighing 150 pounds or the equivalent by weight. An important element in this procedure is the limitation in urinary volume to about 500 for the fourteen hour period when possible. Under these conditions the results obtained show a degree of sensitivity comparing favorably with most kidney function tests.

Objections may arise to the assumption of measuring renal function as a whole, by the capacity to eliminate urea. "It is generally recognized that the kidney does not accomplish work as a unit, but that in forming urine, it eliminates salt, water, uric acid, and urea, by processes which to a certain extent are independent of one another.[22] Thus Myers, Fine

18. McCrae in Osler: Modern Medicine, vol. 3, Philadelphia, Lea & Febiger, 1926.

19. Mosenthal, H. O.: Renal Function as Measured by the Elimination of Fluids, Salt and Nitrogen and the Specific Gravity of the Urine, Arch. Int. Med. **16**:733 (Nov.) 1915.

20. Christian, H. A.: Renal Function in Anemia, Arch. Int. Med. **18**:429 (Oct.) 1916.

21. Stieglitz, E. J.: Disturbances of Renal Function in Pernicious Anemia, Arch. Int. Med. **33**:58 (Jan.) 1924.

22. Mosenthal, H. O.: The Value of Tests for Renal Function in Clinical Medicine, Ohio State M. J. **18**:348 (May) 1922.

and Lough [23] found that in the early stages of impaired renal function uric acid retention preceded that of urea in the blood. Although many functions are performed by the kidney, it is probable that the elimination of nitrogen is one of the most fundamental and the earliest impaired. Recently it has been recognized that in nephrosis and possibly in chronic passive congestion, the retention of water and salt occurs primarily in the body,[24] and that no essential impairment of kidney function is present. DeWesslow [25] has shown that in the absence of edema, the excretion of sodium chloride and nitrogen is strictly parallel, and that both are retained to a like degree in chronic nephritis, while Denis [26] and Loeb and Benedict [27] reported a similar relationship between sulphate and nitrogen.

CONCLUSIONS

1. Following the ingestion of 1 Gm. of urea per 10 pounds of body weight, a characteristic normal curve of blood urea was obtained. The blood urea returned to the control level in fourteen hours.

2. Under similar conditions, a radically different curve was obtained in cases of impaired kidney function. Deviation from the normal occurred even in the early stages of renal impairment. With severely impaired renal function, the deviation from the normal was marked.

3. Early degrees of renal impairment were frequently indicated by marked fourteen hour polyuria (over 750 cc.), although the blood curve remained normal.

4. Based on these results, a simple test of kidney function is described, and the clinical applications are discussed.

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23. Myers, V. C.; Fine, M. S., and Lough, W. G.: The Significance of the Uric Acid, Urea and Creatinin in Nephritis, Arch. Int. Med. **17**:570 (April) 1916.

24. Fishberg, A. M.: The Unitary Nature of Impairment of Renal Function, Arch. Int. Med. **38**:259 (Aug.) 1926. Aldrich, C. A.: A study of the Clinical Course of Generalized Edema, J. A. M. A. **84**:481 (Feb. 14) 1925.

25. DeWesslow, O. L. V.: The Excretion of Chlorides by the Healthy and Diseased Kidney, Quart. J. Med. **19**:53 (Oct.) 1925.

26. Denis, W.: Sulphates in the Blood, J. Biol. Chem. **49**:311, 1921.

27. Loeb, R. F., and Benedict, E. M.: Inorganic Sulphates in Human Blood, J. Clin. Investigation **4**:33, 1927.

AGRANULOCYTOSIS (SCHULTZ) AND THE AGRANULOCYTIC SYMPTOM COMPLEX *

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In 1922, Schultz [1] described a series of cases of a peculiar type of necrosing angina accompanied by a marked absolute and especially granulocytic leukopenia. He asserted that these symptoms were manifestations of a specific disease which he called "agranulocytosis." Since that time, numerous reports of similar cases, at present about 125, have appeared in the literature designated as agranulocytosis or agranulocytic angina (Friedemann [2]). The results of an investigation of 5 cases of agranulocytosis observed between November, 1927, and April, 1928, and a careful and critical study of a large number of records of agranulocytoses induced me to present a discussion of the symptomatology, pathology and differential diagnosis of this disease, because, in my opinion, the term "agranulocytosis" is in a number of cases applied to conditions which differ distinctly from those for which Schultz introduced this name. The following description of the clinical and pathologic aspects of the disease was obtained after the exclusion of all those cases which could not be definitely classified among the essential agranulocytoses.

SYMPTOMATOLOGY

Previous diseases, especially those of the throat, apparently do not have any causative significance. In one case, a commotio cerebri preceded the onset of the disease by several days, and in two cases the patients were under treatment for syphilis. Either the agranulocytosis starts after a more or less protracted period of ill health during which the patients complain that they become easily tired, feel weak and suffer from frequent headache, or, more frequently, it suddenly occurs while the patients are in full health. The symptoms at the onset are high fever of the continued type (from 102 to 104 F.), a pulse of

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1. Schultz: Ueber eigenartige Halserkrankungen, Deutsche med. Wchnschr. **48**:1495, 1922. Schultz and Mirisch: Zur Frage der Anginen mit reaktiver Vermehrung Lymphoider Zellen im Blut, Virchows Arch. f. path. Anat. **264**:760, 1927. Schultz: Seltene Anginaformen und ihre Behandlung, Klin. Wchnschr. **6**:2437, 1927; Zur Frage der Anginen mit atypischem Verlauf, Deutsche med. Wchnschr. **53**:1213, 1927.

2. Friedemann: Ueber Angina agranulocytica, Ztschr. f. Hals-, Nasen- u. Ohrenh. **13**:473, 1926.

high rate and bad quality (from 110 to 140), general malaise, dysphagia and dyspnea. One or several chills may precede or accompany these symptoms. The sore throat makes its appearance at the start or during the first three or four days. A rash, herpes labialis, vomiting and pains in the hypogastric region may occur, but are not common. During the course of the disease, the development of a slight, rarely intense jaundice may take place (in about 50 per cent of the cases reported). Diarrhea also may set in during this stage, but it is a less frequent symptom. The patient usually grows worse rapidly, and death results after coma of from two to seven days' duration in most of the cases. A more chronic course, lasting several weeks, occurs less frequently. Remissions of from a few days sometimes to several weeks may rarely occur. The outcome of the disease is in general fatal. Recoveries are recorded in only a few instances (six). Physical examination shows the following: In the beginning, the tonsils are enlarged and reddened and show white or yellowish plugs. These soon merge to dirty gray or yellow coats, on removal of which an ulcerated surface appears. In the progress of the disease, the tonsillar tissue may become greenish black, and the gangrenous material may slough, sometimes leaving only small stumps of the tonsils. Similar necrosing processes may be found on the pillars, uvula, palate, base of the tongue, pharynx or gums, representing either extensions of the tonsillar alterations or independent processes. Stomatitis is frequently present, and an offensive foetor ex ore is always noticeable. The tongue is usually coated. Hemorrhages from the ulcers or underneath the mucosa rarely occur. The submaxillary and cervical lymph nodes are enlarged and tender. The heart is generally normal, except for increased activity. The blood pressure may be somewhat lowered. In the beginning examination of the lungs gives negative results, but later a condition similar to that present in bronchopneumonia may be noticed. Palpation of the abdomen sometimes reveals tenderness in the epigastric region, especially over the gallbladder. The liver and the spleen, the latter more often than the former, are frequently enlarged. Ulcerations similar in appearance to those in the mouth may be observed in the anal region and at the vulva, vagina and uterine cervix. Tests for hemorrhagic diathesis (Rumpel-Leede) give negative results. Petechial hemorrhages in the skin are rare and scanty. Examination of the urine for albumin always gives slightly positive results. The sediment shows hyaline and granular casts, leukocytes and erythrocytes. Ehrlich's test and the test for sugar are negative. Urobilin and urobilinogen may be present in the patients who have jaundice. The bacteriologic examination of the throat does not render results of any special significance. Staphylococci, streptococci and pneumococci are the micro-organisms usually found. Fusospirillosis was noted in about 10 per cent of the cases. The most important observations are made during examination of the blood.

There is a considerable decrease in the number of the leukocytes, which becomes more and more marked toward death (from 1,500 to 100 in 1 c. mm.). The granulocytic cells evidently decrease first and may finally disappear completely. Immature forms of these cells are not present, but degenerative forms may be observed. Besides the absolute granulocytic leukopenia, there is also a lymphocytic decrease which follows closely that of the granulocytic cells. The monocytes are sometimes temporarily somewhat increased in number. The oxydase reaction is always negative for these cells. The red blood cell picture (erythrocytes and hemoglobin) is normal or shows only minor changes. The number of the thrombocytes is either normal or increased. Pathologically, large forms are not rarely observed. The coagulation time and bleeding time are normal. The Widal test and the Wassermann test are negative, except in patients previously infected with the corresponding diseases. Blood cultures are more likely to be negative than positive (in about 10 per cent). Different bacteria may be obtained on culture (hemolytic and nonhemolytic streptococci and staphylococci, *Streptococcus viridans, Bacillus pyocyaneus, Bacillus acidi-lactici* and *Bacillus coli).* Positive cultures are rarely obtained during the first days of the disease.

PATHOLOGY

The necrosing processes in the mouth may vary considerably. Only a few superficial ulcerations may be present on the tonsils, or there may be extensive, multiple, deep gangrenous destructions involving the esophagus and the larynx. They are sometimes surrounded by a zone that is a wine red in color. Microscopically, the bottom of the ulcers is composed of three layers. A necrotic, granular material intermingled with numerous bacteria forms the uppermost layer. In the next one underneath, which usually extends into the muscle tissue, the tissue is also necrotic but the cellular outlines are preserved. Streaklike accumulations of bacteria may be found in the intercellular spaces. The vessels in this region are thrombotic and contain hyaline or fibrinous material or clotted blood in which the absence of leukocytes is striking. In the still deeper edematous layer, areas of living and necrotic tissue alternate. Smaller and larger accumulations of lymphocytes and plasma cells may be present in varying number.

Subpleural hemorrhages are frequently observed. A fibrinous exudate may cover the pleura in places in which dark red, solid, irregular small foci are present in the lung. Such areas are regularly found, especially in the lower lobes which, moreover, may show hypostatic hyperemia. The capillaries of these solidified parts are hyperemic; the alveoli are filled with red blood corpuscles and in places with bacterial masses. The adjacent alveoli contain an albuminous or fibrinous material. The absence of leukocytes in these foci is remarkable.

Subpericardial and subendocardial hemorrhages are frequently seen.

Ulcerations similar to those found in the mouth are frequently present in some or all parts of the digestive tract, especially in the esophagus and intestine, where they are sometimes extensive. The intestinal lymph follicles are swollen, and the mucosa covering the Peyer's patches may be found ulcerated, as in typhoid fever. The stomach shows hemorrhages and erosions more frequently than small single or multiple ulcers.

The liver is usually slightly enlarged and shows evidence of cloudy swelling. Microscopically, varying degrees of fatty degeneration, occasionally multiple focal necroses and in general, an increase and proliferation of Kupffer's cells exist. The bile capillaries frequently contain bile casts and the liver cells bile pigment. Interstitial lymphocytic infiltrations are sometimes observed in scanty to moderate number.

A minor swelling of the spleen is common. It is dark red and moderately firm, but never soft as in septicemia. The lymph follicles are not prominent on the cut surface. The sinuses are well filled with erythrocytes and proliferated reticulo-endothelial cells and lymphoid cells. Cells positive for oxydase are in general completely absent or are only scantily present. Hemosiderin is often found in large phagocytic cells. The lymph follicles are small and atrophic, especially the germinative centers, which are sometimes composed of mature lymphocytes. The proliferated reticulo-endothelial cells may outnumber the lymphoid cells in the spleen. Small anemic necroses are rarely observed.

The submaxillary, cervical, peribronchial and mesenteric lymph nodes are in general enlarged. They may occasionally contain hemorrhages. The microscopic examination reveals atrophy of the lymph follicles as in the spleen and a proliferation of the reticulo-endothelial cells.

The kidneys usually show signs of cloudy swelling. Numerous red points the size of a pinhead are found beneath the capsule in the cortex; they represent swollen, hyperemic glomeruli. On the cut surface, the cortex and medulla are not well demarcated. The tubular epithelium is in general markedly degenerated, and the tubular lumina are frequently filled with casts. Petechial hemorrhages are rarely found in the renal pelvis.

The marrow in the femur is either completely or partly composed of red marrow. It is mainly made up of lymphoid cells. Erythroblasts and megakaryocytes are present in normal numbers. Cells positive for oxydase are rarely seen, and then they are only scanty.

Ulcerations of the same appearance as those described for other organs are frequently observed on the vulva, vagina and cervix. Sometimes, an intra-uterine hemorrhage is present.

GENERAL ASPECTS

Agranulocytosis is by far more frequent in women than in men. According to my investigations, the ratio is 3.5 : 1. The disease affects mainly those of middle age (from 30 to 50), but occurs also in younger and in older persons. If correct conclusions can be drawn from the origin of the reports, the disease seems to be prevalent in Germany and Austria. The country next in frequency is the United States, with Canada next. Single cases are described by Dutch, French and Italian authors. A reason for this peculiar local distribution is not evident, and suggestions cannot be offered. The disease is apparently not contagious.

THERAPY

Diphtheria antitoxin, arsphenamine locally and intravenously, methenamine, mercurochrome-220 soluble, an organic iodine compound-casein, alcohol, various silver compounds, and acridine dyes and many other local caustics and disinfectants were used without any favorable effects. Temporary improvement was seen after repeated transfusions of large amounts of blood and recovery after administration of polyvalent antistreptococcus serum and roentgen-ray irradiation of the long bones with stimulating doses. The spleen was irradiated without success.

ETIOLOGY

The cause of "agranulocytosis (Schultz)" is still unknown. The theories concerning its etiology as supported by the different authors may be classified into three groups:

1. Friedemann [2] asserted that it is the result of an endocrine disturbance.

2. Mouzon [3] and Roch and Mozer [4] were inclined to place it in relation to the acute leukemias.

3. The majority of the workers in this subject assert that it is an infectious disease and represents a septicemia with an atypical reaction of the hematopoietic system (Feer,[5] Weiss [6]) due either to bacteria with a special affinity and toxicity to the granulocytic

3. Mouzon: Agranulocytose, Presse méd. **34**:1269, 1926.

4. Roch and Mozer: Angina agranulocytotica, Presse méd. **34**:1171, 1926.

5. Feer: Beitrag zur Frage der Agranulocytose, Schweiz. med. Wchnschr. **56**:551, 1926.

6. Weiss: Ueber die gegenseitigen Beziehungen zwischen Schultzschem Symptomkomplex (Mucositis necroticans agranulocytica), akuter Leukämie und septischem Infekt, Wien. Arch. f. inn. Med. **14**:303, 1927.

system (Zadek,[7] David,[8] Sternberg,[8] Pelnar[9]) or to an atrophy or hypoplasia of this organ because of the presence of a septic infection (Zikovsky,[10] Türk[11]). According to these investigators, the bacterial cause is either nonspecific (Ehrmann and Preuss[12]) or specific (streptococci [Zikovsky], *Bacillus pyocyaneus* [Lovett[13]] fusospirillosis [Cannon[14]]). Schultz[1] and Reye and Wohlwill[15] simply stated that they consider it a disease sui generis and supported their standpoint by emphasizing the uniformity of the pathologic symptoms.

DIFFERENTIAL DIAGNOSIS

Numerous diseases may more or less resemble agranulocytosis in one or the other stage. They may be classified as follows:

1. Diseases showing the agranulocytic symptom complex and necrosing processes in the mouth.

(*a*) Influenza and typhoid fever differ from essential agranulocytosis in their course and their bacteriologic and pathologic manifestations in spite of the existence of a certain resemblance in acute cases in the beginning.

(*b*) Septicemia and its special modifications, such as Werlhof's disease, differ from agranulocytosis in the presence of a general hemorrhagic diathesis, abscesses, a primary infectious focus, immature forms of granulocytes in the blood, thrombopenia, secondary anemia, frequently positive blood culture, hyperleukocytosis in the spleen, fat marrow in the long bones, and myeloid proliferations in the bone marrow.

(*c*) Acute leukopenic leukemias and aleukemic leukemias can be distinguished from agranulocytosis by the existence of a generalized hemorrhagic diathesis, secondary anemia, thrombopenia, lengthened bleeding time, tenderness of the lower part of the sternum and charac-

7. Zadek: Zur Frage der "Agranulocytose," Med. Klin. **21**:688, 1925.

8. Quoted by Zadek (footnote 7).

9. Pelnár: Malignant Leukopenia, Čas. lék. česk. **63**:1653, 1924; abstr., J. A. M. A. **84**:74 (Jan. 3) 1925.

10. Zikovsky: Zur Kenntnis der Agranulocytose, Wien. med. Wchnschr. **77**:589, 1927; Zur Frage der Agranulocytose, Wien. klin. Wchnschr. **40**:1376 and 1420, 1927.

11. Türk, quoted by Weiss (footnote 6).

12. Ehrmann and Preuss: Ueber Leukopenie und Schwund der Granulocyten bei Sepsis (Agranulocytose), Klin. Wchnschr. **4**:267, 1925.

13. Lovett, R. W.: Agranulocytic Angina, J. A. M. A. **83**:1498 (Nov. 8) 1924.

14. Cannon: Some Unusual Dermatoses; Agranulocytic Angina, South. M. J. **20**:141, 1927.

15. Reye and Wohlwill, quoted by Leuchtenberger: Ueber Agranulocytose, Klin. Wchnschr. **5**:1492, 1926.

teristic pathologic alterations such as leukemic infiltrations in the liver, spleen, lymph nodes and kidney.

(*d*) Aleukia (Ehrlich) is also characterized by a general hemorrhagic diathesis, thrombopenia, secondary anemia and lengthened bleeding time, such as occurs in some of the diseases already mentioned. It resembles agranulocytosis in that atrophy of the total myeloid tissue occurs but with inclusion of megakariocytes. Moreover, myeloblasts are still present in the atrophic bone marrow.

2. Diseases showing an agranulocytic symptom complex but without gangrenous ulcers in the mouth.

To this group belong various diseases such as the abdominal type of Hodgkin's disease (Jaffé,[16] Feer [5]), pernicious anemia, carcinosis of the bone, miliary tuberculosis, poisonings with arsenic compounds (arsphenamine) and benzene and diseases in which roentgen-ray irradiation (especially in the treatment for chronic lymphatic and myeloid leukemia) and thorium X are employed.

3. Diseases showing similar necrosing processes in the mouth, but without an agranulocytic symptom complex.

(*a*) Diphtheria, especially its fulminant type, in its general and local symptoms sometimes closely resembles agranulocytosis, but it is different in hematologic, bacteriologic and pathologic respects. In seven of the cases reviewed, it was specially stated that the preliminary diagnosis was diphtheria.

(*b*) Vincent's angina may also show local alterations similar to those found in agranulocytosis, but here also the differential diagnosis rests on the general condition of the patient, the condition of the blood, the good prognosis and the pathologic alterations.

(*c*) Monocytic angina (Schultz [1]), like Vincent's angina, usually occurs at an earlier age (from 12 to 27) than agranulocytic angina. In this disease necrosis is restricted to the tonsils, and a generalized enlargement of the lymph nodes and of the liver and spleen occurs. The latter remains persistent for several months after recovery. The blood shows an increased number of white cells, with an absolute increase of the monocytes (from 6 to 78 per cent). Jaundice is never present, nor are hemorrhages observed. Females are not affected more than males, and the prognosis is in general good. Few fatalities are reported. The disease is apparently contagious and has a protracted course.

4. Other diseases which represent differential diagnostic possibilities.

In the beginning, agranulocytosis may sometimes resemble acute cholecystitis in its abdominal manifestations. But besides the state of

16. Jaffé: Agranulocytärer Symptomkomplex bei Hodgkinschem Lymphogranulom, München. med. Wchnschr. **73**:2012, 1926.

the throat and the blood, the discrepancy between the unfavorable general condition of the patient and the relatively light local symptoms in agranulocytosis will point to the correct diagnosis.

COMMENT

1. Essential agranulocytosis (Schultz) can be differentiated by its clinical and pathologic symptoms from diseases showing a secondary agranulocytic symptom complex.

2. The demonstration of bacteria in the blood of patients with agranulocytosis is not proof of the infectious character of the disease, because bacteria are also frequently found in later stages of leukemias and their presence is due to invasion of the body through the areas of necrosis.

3. Agranulocytosis is apparently not contagious.

4. Its prognosis is bad, but not absolutely infaust.

5. The disease may be confused with fulminant diphtheria if an examination of the blood is not made.

6. The unknown noxa injures not only the granulocytic system but the lymphatic system, as is evident from the marked absolute decrease of these cells in the blood and in the lymphatic organs. The reticulo-endothelial system apparently undergoes a compensatory hyperplasia.

INGESTED CREATINE

ITS UTILIZATION AND RATE OF EXCRETION BY ARTHRITIC AND NORMAL SUBJECTS *

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When creatine is administered orally to men and animals, a portion of it may be excreted in the urine apparently unchanged, the remainder being retained by the organism. If the quantity of creatine ingested is small, as was the case in some of Folin's early experiments [1] and in a recent experiment of Rose, Ellis and Helming,[2] none will appear in the urine. With larger doses an appreciable quantity is excreted, but some is always retained, normally. The efficiency of the body in utilizing a given dose of creatine decreases if the administration of creatine is continued from day to day. This was clearly shown in the experiments of Benedict and Osterberg on dogs [3] and in those of Chanutin [4] and Rose, Ellis and Helming [2] on men, in which creatinuria appeared and became more decided as the creatine feeding was persisted in. Chanutin [4] has pointed out that in respect to their utilization, there is a similarity between creatine and dextrose. Both substances may be completely utilized when fed in small amounts, but the capacity of the body to retain either may be readily overcome if, instead of feeding a small quantity, a massive dose is employed.

The response of the body to a single massive dose of dextrose has been extensively studied, and dextrose feeding has been used as a test to detect defective sugar metabolism. In a similar manner it may be possible to detect an abnormal creatine metabolism by observing the ability of the body to utilize a large dose of creatine. In conditions of frankly deranged creatine and creatinine metabolism, the capacity to retain administered creatine is greatly diminished as was shown by

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* The work here reported is a part of a series of studies on chronic arthritis by Ralph Pemberton, M.D., of Philadelphia, in collaboration with Robert B. Osgood, M.D., of Boston. The expenses of this investigation were defrayed by contributions from various sources, including a number of patients.

1. Folin, O.: Hammarsten's Festschr., 111: Upsula, 1906.

2. Rose, W. C.; Ellis, R. H., and Helming, O. C.: J. Biol. Chem. **77**:171 (April) 1928.

3. Benedict, S. R., and Osterberg, E.: J. Biol. Chem. **56**:229 (May) 1923.

4. Chanutin, A.: J. Biol. Chem. **67**:29 (Jan.) 1926.

Gibson and Martin,[5] who studied a case of progressive pseudohypertrophic muscular dystrophy with a creatinuria and a low creatinine excretion. They found that creatine when given to this patient, even in small amounts (0.5 Gm.), was promptly and completely eliminated, chiefly as creatine.

The following experiments were undertaken to compare the utilization of creatine administered as a single large dose to patients with proliferative arthritis, on the one hand, with the utilization of the same quantity by normal healthy subjects, on the other. A difference in the two groups in their ability to utilize creatine when so given would be suggestive. Pemberton and Buckman[6] found slightly high values for creatine in the blood in half of a series of fifty cases of arthritis studied by them in the army. The frequency of the occurrence of myositis with chronic arthritis made it also seem not unlikely that the arthritic patient might exhibit a peculiar response to ingested creatine. Levene and Kristeller[7] found a low rate of creatine catabolism in pathologic conditions involving the muscular system.

In the present experiments, the rate of excretion of the unutilized creatine has also been studied. Examination of the literature shows that studies have not been reported heretofore in which the urine has been analyzed at frequent intervals after creatine feeding to determine how rapidly the body excretes that part of the creatine which is not retained.

EXPERIMENTAL PROCEDURE

Seven women patients with active proliferative arthritis and seven persons in apparent good health, four women and three men, served as subjects for these feeding trials. Five of the women in the arthritic group were middle aged and two were young. With the exception of one elderly woman, the normal subjects were all young—interns, laboratory and hospital workers.

Each experiment consisted of a three day period, during which the following procedure was carried out. A preliminary twenty-four hour collection of urine was made during the first day. On the morning of the second day, 10 Gm. of creatine, dissolved in a glass of hot, sweetened lemonade, was given orally. The taste of the creatine was almost undetectable in the presence of the lemon juice, and nausea or gastric symptoms were not encountered.

The creatine employed was a high grade commercial product,[8] which was found on analysis to be free from creatinine and to have a melting point, nitrogen content and water of crystallization close to that calculated for creatine.

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5. Gibson, R. B., and Martin, F. T.: J. Biol. Chem. **49**:319 (Dec.) 1921.

6. Pemberton, R., and Buckman, T. E.: Studies on Arthritis in Army Based on 400 Cases: Studies in Relation of Creatine Metabolism to Arthritis, Arch. Int. Med. **25**:335 (April) 1920.

7. Levene, P. A., and Kristeller, L.: Am. J. Physiol. **24**:45, 1909.

8. We are indebted to Mr. C. Broxton Valentine, chemist of Valentine's Meat Juice Company of Richmond, Va., for this product.

During the twenty-four hours following the ingestion of creatine, collections of urine were divided into two three hour periods, one six hour period and one twelve hour period. A twenty-four hour collection of urine was made on the third day of the experiment.

During these three days, meats, gravies and soups prepared from meat stock were excluded from the diet. Other dietary restrictions were not imposed.

The following methods were used in the analysis of the specimens of urine, all of which were preserved with toluene. Folin's colorimetric method was used for the determination of creatinine,[9] employing as standard a solution of creatinine in tenth normal hydrochloric acid. Urinary creatine was converted into creatinine by heating in the autoclave with normal hydrochloric acid as directed by Benedict and Myers.[10] The total nitrogen was determined by Folin's method.[9]

TABLE 1.—*The Utilization of 10 Gm. of Creatine by Normal and Arthritic Subjects*

No.	Condition	Sex	Creatinine Coefficient	Creatine * Excreted 24 Hours Before Feeding Gm.	Extra * Creatine Excreted After Feeding Gm.	Creatine * Retained Gm.	Creatine Retained Per Cent
7	Arthritis.....	F	10.7	0	4.81	2.77	37
5	Arthritis.....	F	10.9	0	2.91	4.67	62
10	Arthritis.....	F	12.5	0.09	3.61	3.97	52
4	Arthritis.....	F	14.6	0.19	5.90	1.68	22
9	Arthritis.....	F	14.7	0.19	4.95	2.43	32
3	Arthritis.....	F	15.1	0.02	5.42	2.16	29
6	Arthritis.....	F	15.7	0.05	4.33	3.25	43
8	Normal.....	F	13.9	0.01	3.52	4.06	54
11	Normal.....	F	17.0	0.04	3.65	3.93	52
12	Normal.....	F	20.4	0	4.61	2.97	39
2	Normal.....	M	23.8	0	2.45	5.13	68
1	Normal.....	M	24.1	0.03	3.35	4.23	56
13	Normal.....	F	25.2	0	4.47	3.11	41
14	Normal.....	M	26.5	0	3.43	4.15	55

* Creatine values expressed as creatinine in grams; 10 Gm. creatine = 7.58 Gm. creatinine.

THE UTILIZATION OF INGESTED CREATINE

The data showing the utilization of creatine by the arthritic and normal subjects, as revealed by urinalysis, are given in table 1. The results are arranged for both groups in order of increasing creatinine coefficients. It will be seen that wide individual variations in ability to retain ingested creatine were encountered, the percentage of the utilization of creatine ranging in the arthritic patients from 22 to 62 and in the normal subjects from 39 to 68. The arthritic patients were, as a whole, slightly less efficient in their ability to retain creatine. The subjects in this group, however, had lower creatinine coefficients than the normal persons, and for this reason might not be expected

9. Folin, O.: Laboratory Manual of Biological Chemistry, ed. 3, New York, D. Appleton & Company, 1922.

10. Benedict, F. G., and Myers, V. C.: Am. J. Physiol. **18**:397, 1907.

to have so great a capacity for retaining creatine as the normal subjects with a high creatinine coefficient and hence, perhaps, a larger storage reservoir for creatine. When the ratio of creatine retained to creatinine coefficient is considered, there do not appear to be differences between the two groups in their respective abilities to retain ingested creatine. It should be pointed out, however, that a clear relationship was not observed between the creatinine coefficient and the utilization of creatine. The largest retentions of creatine were not always associated with high coefficients, nor did those subjects with the lowest creatinine coefficients retain the smallest amount of the creatine.

As will be mentioned later, small amounts of unutilized creatine were being excreted by the arthritic patients, in contrast to the normal

TABLE 2.—*Excretion of Creatinine, Nitrogen and Water Following the Ingestion of 10 Gm. of Creatine*

	Creatinine (Gm.)				Total Nitrogen (Gm.)			Volumes (Cc.)		
No.	1st Day	2nd Day	3rd Day	Increase on 2nd Day	1st Day	2nd Day	3rd Day	1st Day	2nd Day	3rd Day
5	0.60	0.69	0.61	0.09	4.10	4.73	4.25	1930	1605	2410
3	0.65	0.75	0.54	0.10	5.26	5.98	5.93	1790	1455	1220
10	0.66	0.76	0.66	0.10	6.59	6.92	6.05	1665	1425	1190
4	0.73	0.67	0.76	—0.06	7.41	6.92	6.78	1575	1435	1315
6	0.76	1.06	0.87	0.30	6.72	8.09	6.08	1615	1945	2050
9	0.79	0.88	0.77	0.09	4.83	6.71	4.83	2720	2193	2595
7	0.80	0.95	0.83	0.15	7.80	8.20	5.90	3295	3565	1350
8	0.92	1.02	0.95	0.10	6.54	6.99	6.44	1030	1080	1100
11	1.03	1.25	1.01	0.23	7.54	11.25	8.97	1425	2038	1572
12	1.03	1.31	1.25	0.28	6.39	7.10	5.21	1365	1745	1605
13	1.59	1.48	1.37	—0.11	10.28	11.13	8.47	1020	1383	1524
2	1.72	2.01	1.70	0.29	10.77	10.80	10.10	1105	1095	790
14	2.13	2.17	....	0.04	11.04	14.11	.....	640	1525	....

subjects, during the second twenty-four hours following creatine feeding. If this had continued longer, the calculated values for utilization of creatine in table 1 would be a little high.

The data for the excretion of water, creatinine and nitrogen during this experimental regimen are recorded in table 2.

During the day of creatine feeding there was a distinct increase in the excretion of creatinine in all but two of the experiments. This increase was present only on the day when creatine was taken, and on the following day urinary creatinine had usually returned to the excretion level of the fore period.

The figures on the excretion of nitrogen are of limited value only, in view of the fact that the intake of nitrogen was not necessarily a fixed quantity during the three days of the experiment. After deducting the nitrogen contained in the urinary creatine, less nitrogen was excreted

in most of the experiments on the day that creatine was administered than on the other two days.

The twenty-four hour volumes did not usually show any great variation during the course of the experiment.

THE RATE OF EXCRETION OF UNUTILIZED CREATINE

The creatine content of the urine during the periods following creatine feeding is given in table 3. It was found that the fraction of ingested creatine which the body did not utilize was promptly excreted and that in most cases by the end of six hours most of the unretained creatine had appeared in the urine.

With one exception, the peak of excretion of creatine by the normal subjects occurred during the first three hours after the creatine was ingested. From this peak, excretion of creatine diminished rapidly

TABLE 3.—*The Rate of Excretion of Creatine Following the Administration of 10 Gm. of Creatine* *

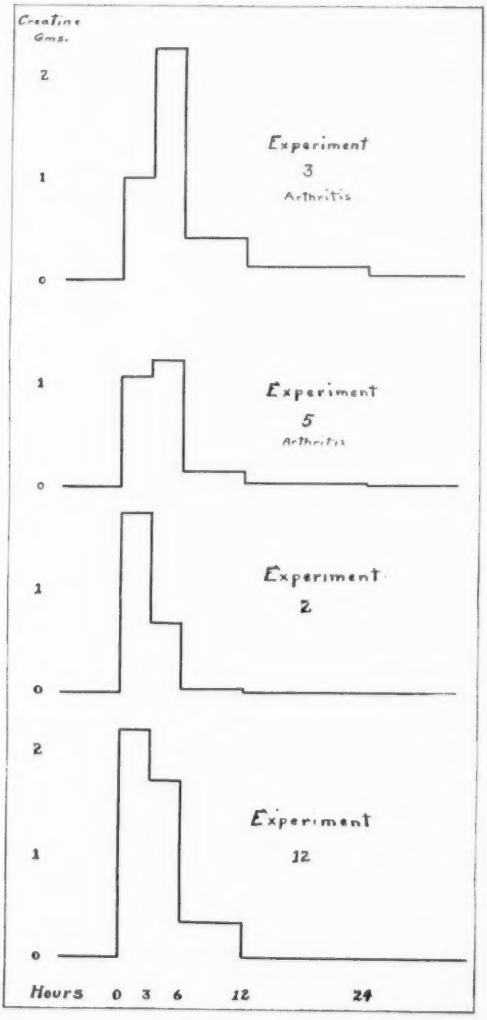
	Normal Subjects						Arthritic Patients						
Number	2	8	11	12	13	14	3	4	5	6	7	9	10
24 hr. fore period.	0	0.01	0.04	0	0	0	0.02	0.19	0	0.05	0	0.19	0.07
0- 3 hrs.	1.73	1.92	0.93	2.21	2.76	2.78	1.01	1.26	1.09	1.57	1.04	2.04	0.96
3- 6 hrs.	0.68	1.11	1.42	1.72	1.04	0.65	2.29	1.92	1.24	1.74	2.38	1.22	1.17
6-12 hrs.	0.04	0.50	0.51	0.68	0.49	0	0.85	1.78	0.33	0.60	0.84	0.76	....
12-24 hrs.	0	0	0.37	0	0.18	0	0.68	0.60	0.11	0.31	0.35	0.53	....
24-48 hrs.	0	0	0.50	0	0	0	0.63	0.72	0.14	0.21	0.19	0.48	0.52

* Creatine values expressed as creatinine in grams. Ten Gm. creatine $\simeq$ 7.58 Gm. creatinine.

and usually was completed by the end of twelve hours. In the arthritic group a definite lag in the excretion of creatine has been found. In this group, the peak of excretion was usually not reached until the second period, that is, during the third to sixth hour after the administration of creatine. The creatinuria lasted longer in these subjects than in the controls, and in all seven of the experiments on arthritis, extra creatine was being excreted during the second twenty-four hours. A similar delay in disposal of the ingested creatine was encountered only once among the supposedly healthy subjects.

In the accompanying chart the rate of the excretion of creatine, per three hours, for four subjects, two normal persons and two arthritic patients, has been plotted.

The delay in excretion of creatine and the persistence of the induced creatinuria found among the arthritic patients can hardly be attributed to a failure on the part of these subjects to store creatine, when the total utilization data are considered. Evidence has not been found in these experiments to indicate that the patients in this group are less



The three hourly rate of excretion of creatine following the administration of 10 Gm. of creatine.

capable than the subjects in the normal group in their ability to retain creatine when it is fed to them in a large dose.

Consideration must be given to the possibility of an impaired renal function in those persons who showed a delay in the excretion of creatine. Pemberton and Foster [11] found normal values for urea and nonprotein nitrogen in the fasting blood in patients with arthritis. They detected in their subjects a slight delay in the excretion of water, nitrogen and salt, following the Mosenthal test meal, but were unable to conclude that any marked dislocation of renal function is present in chronic arthritis. If the delayed excretion of creatine is to be ascribed to renal insufficiency, which seems doubtful, there is then available in creatine feeding a delicate test for the function of the kidney.

The observation that in all the arthritic subjects and in one of the normal subjects a delay in the excretion of creatine follows massive administration of creatine can only be stated as fact at this time without attempting to give an explanation of it. It is, however, of considerable interest to recall that the arthritic patient frequently exhibits a delay in disposal of sugar following ingestion of dextrose, despite the fact that there is no evidence of failure of combustion of dextrose by him. This delayed "sugar removal" has been definitely referred in the arthritic patient to a defective blood supply to those parts actively concerned in the removal of sugar from the blood.[12] The somewhat similar delay in disposal of creatine, when fed in large amounts, may result from some factor similar to that causing the delay in the disposal of sugar.

SUMMARY

The utilization of creatine, when administered orally in a single 10 Gm. dose, has been studied.

The rate of excretion of creatine and the persistence of the creatinuria induced by the ingestion of this amount of creatine have also been determined.

Individual variations of considerable magnitude were found in the amounts of the ingested creatine utilized. When the differences in creatinine coefficients are considered, the patients in the arthritic group proved to be as efficient as the subjects in the normal group in their ability to retain creatine.

11. Pemberton, R., and Foster, G. L.: Studies on Nitrogen, Urea, Carbon Dioxide Combining Power, Calcium, Total Fat and Cholesterol of Fasting Blood, Renal Function, Blood Sugar and Sugar Tolerance, Arch. Int. Med. **25**:243 (March) 1920.

12. Pemberton, R.; Cajori, F. A., and Crouter, C. Y.: Influence of Focal Infection and Pathology of Arthritis, J. A. M. A. **85**:1793 (Dec. 5) 1925.

The rate of excretion of the excess creatine was slower, however, in the arthritic patients than in the normal subjects. The peak of excretion was not reached as soon after the creatine was administered, and the creatinuria persisted longer in the arthritic patients than was the case in all but one of the normal control subjects.

The fact that a delay has been encountered in arthritis in the disposal of ingested dextrose suggests that the factors responsible for the slow rate of excretion of creatine may be similar to those which cause the delay in the removal of ingested dextrose. In the case of dextrose, the delay in the removal of sugar has been referred to a disturbance of peripheral blood flow in patients with arthritis.

THE EFFECTS OF SERUMS FROM NORMAL AND FROM ANEMIC PERSONS ON THE GROWTH OF SEEDLINGS*

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The impetus given to the study of pernicious anemia by the discovery of Minot and Murphy [1] that a remission could be induced and maintained by a diet rich in liver or an effective liver extract has reopened several problems in connection with the disease. The presence of toxic substances in the blood stream as an etiologic factor has been considered by Macht [2] although, as Minot pointed out,[1] these substances could be a result of the altered metabolism produced by the disease rather than a causative factor. In a series of studies on the effect of serums taken from patients with pernicious anemia and other serums on the growth of lupine seedlings, Macht [3] found that the average coefficient of growth in Shive's solution which contained normal blood serum was 75 per cent of the growth of the seedlings as compared with similar growth in Shive's solution alone. On the other hand, the indexes of growth obtained with specimens of blood from patients with pernicious anemia varied from 28 to 51 per cent, giving an average coefficient of 44 per cent. This procedure was suggested as a method of differential diagnosis; when a coefficient of growth was obtained of 50 per cent or less, as compared with a normal index of from 50 to 75 per cent, the results were in favor of pernicious anemia. The hydrogen ion concentration of the serums did not play a rôle in explaining a selective toxicity of the toxin for plants. The serums of patients with secondary anemia were practically nontoxic. It was possible to detoxify the serum of patients with pernicious anemia by exposure of the serum in a quartz tube to

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1. Minot, G. R., and Murphy, W. P.: A Diet Rich in Liver in the Treatment of Pernicious Anemia, J. A. M. A. **89**:759 (Sept. 3) 1927.

2. Macht, D. I.: Etiology, Diagnosis and Treatment of Pernicious Anemia, J. A. M. A. **89**:753 (Sept. 3) 1927.

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ultraviolet light. This phenomenon was enhanced by the addition of sensitizers as tetrabromfluorescein or similar substances. After a transfusion of blood from a patient with pernicious anemia, the phytotoxic index approached the normal. A similar effect was noted after the patient had been treated with a diet rich in liver, although the effect was much less marked, and the detoxification was not complete. A decrease in the toxicity of the blood was noted after patients with pernicious anemia were irradiated with ultraviolet light, with the possibility of a slightly more definite action after the treatment of the patients with eosin as a sensitizer. Treatment wtih organic mercurials also caused a detoxification of the blood serums. Macht's work points to the presence of a definite substance in the blood serums of patients with pernicious anemia, which he believes to be related etiologically to the disease and which is phytotoxic.

To determine the value of this test in the differential diagnosis, as well as to note any changes which feeding of liver would bring about, the blood of twenty-five normal persons as well as thirty specimens of blood of fifteen patients with pernicious anemia in various stages of the disease was studied, together with some specimens from seven patients with other types of anemia. (Sixty-two serums were tested, using 1,260 seedlings.)

METHOD OF STUDY

The blood serums were examined, and the technic outlined by Macht[3] was used. Briefly, the procedure was as follows: *Lupinus albus* seeds of approximately the same size were soaked in a jar of clean tap water overnight. On the following morning these seeds were taken from the jar of tap water and placed with the hilum down in a pot of moistened, clean moss of the genus *Sphagnum*. After covering these seeds with this moss, they were placed in the dark at a temperature of 68 F., and were left from two and a half to three days, according to the growth exhibited. On reaching the proper length the seedlings were set up in the following manner: Hard glass test tubes (1.5 by 20 and a content of 30 cc.) were used; these were placed in holes in a wooden block holding fifteen test tubes in an upright position. Shive's solution R5C2 (10.4 cc. of 0.5 molar solution of calcium nitrate; 30 cc. of 0.5 molar solution of magnesium sulphate; 36 cc. of 0.5 molar solution of monopotassium acid phosphate; 1 liter of distilled water) was made of Baker's chemically pure analyzed chemicals.[4]

Blood was obtained from patients and from members of the staff of the Simpson Memorial Institute one hour before it was used. The blood was collected in sterile test tubes from patients before they had eaten breakfast; neither antiseptics nor anticoagulents were added. After the serum had separated from the clot, the material was centrifugalized and transferred with sterile pipets to sterile test tubes. A 1 per cent solution from each serum was made in Shive's solution. For each observation, a set of fifteen seedlings was used for each solution. The 1 per cent solution was mixed and shaken well in flasks and then poured into the test tubes to within from 1 to 1.5 cm. from the top (27 cc. of solution). For each

4. Shive, W. J.: A Study of Physiological Balance in Nutrient Media, Physiological Research **1**:327 (Nov.) 1915.

experiment, serum from a normal person was used as a control, as well as seedlings in Shives solution alone. When these solutions were prepared, seedlings of *Lupinus albus* of approximately the same length were selected. These seedlings were carefully measured, from the darkened region of demarcation of the root and stem to the tip of the root, on a centimeter ruler fastened to a piece of soft wood. This measurement was recorded, and the seedlings were placed in the proper test tube. The seedling was held in position by hanging it over the edge of the test tube by the cotyledons and supporting it by pledgets of cotton. The p_H values were determined for the solutions colorimetrically. Shives solution averaged p_H 4, and the 1 per cent serum solution averaged p_H 4.4; there was some little variation.

The tubes containing the seedlings were then placed in the dark at a temperature of 68 F. At the end of twenty-four hours, measurement of the roots was again made and recorded. Similar measurements were recorded at the end of another twenty-four hours (forty-eight hours). At the end of forty-eight hours the p_H values were determined. There was little variation for the p_H values for the different solutions (Shive's p_H 4; 1 per cent solution p_H from 4.3 to 4.4).

Since the increase in growth was important, this observation was made for each seedling at twenty-four and forty-eight hour periods. These figures for a given set of observations were added together and the average found (in centimeters) for the seedlings in a given solution. For each solution the increase in centimeters of the length of the root for twenty-four and forty-eight hours was compared to the increase in growth in Shive's solution and that in the serum of normal persons. The percentage growth in each solution was also calculated. The growth in centimeters in Shive's solution was taken as 100 per cent and that in the serum solutions calculated as a percentage of this. This procedure was done separately for each set of observations.

RESULTS

The accompanying tables give the serums employed, the growths of the seedlings in centimeters and the percentage growth for twenty-four and forty-eight hour periods in different solutions used.

COMMENT

From the foregoing observations an average of growth for twenty-four hours was established for seedlings in a normal blood serum. To establish this average, fifteen seedlings were grown in each of the twenty-five normal serums, making a total of 375 seedlings. The average coefficient of growth for the seedlings in normal serums was 76.5 per cent as compared to that in Shive's solution. Several of these twenty-five normal serums were taken from men before they had eaten breakfast in order to determine whether or not the coefficient of growth would be altered by processes of digestion. Comparisons failed to show a significant difference.

It is to be noted, however, in establishing this average for growth in normal serum, that there was a wide range of variations in individual sets of serums (from 50 to 97 per cent).

In the thirty specimens of serum taken from fifteen patients with pernicious anemia, the indexes of growth obtained varied from 59 to 94.3 per cent, with an average of 76.6 per cent. This average was obtained from patients with pernicious anemia per se without regard to treatment with liver or to clinical conditions. If, from this group, those patients (five in all) who had not had treatment with liver at the time the serum was obtained were separated, the indexes of growth varied from 60.1 to 86.9 per cent with an average of 75.4 per cent.

TABLE 1.—*Serums, Growth of Seedlings and Percentage Growth in Twenty-Four and Forty-Eight Hour Periods*

Case No.	Sex	Day of Observation	Red Blood Cells Millions per Cubic Millimeter	Per Cent Hemoglobin	Bilirubin Mg. per Hundred Cubic Centimeters	Liver or Liver Extract	Clinical Condition	24 Hours Growth of Roots in Percentage of Growth in Shive's Solution
1	M	1	3.5	53	0.8	+	Mod. sick	67.4
		5	4.7	83	...	+	Good	93.8
		11	5.21	65	1.1	+	Good	73.3
2	M	1	1.8	35	2.3	0	Very sick	81.0
		17	3.3	53	0.9	+	Good	85.6
		31	4.23	64	0.9	+	Good	93.4
3	F	1	2.3	50	0.7	+	Very sick	71.2
		8	3.1	49	0.4	+	Improved	65.3
		15	4.0	63	0.3	+	Good	84.5
4	M	1	1.04	26	1.2	0	Very sick	60.1
		23	2.1	44	1.1	+	Good	75.3
		31	2.9	56	0.5	+	Good	59.7
5	M	1	0.78	18	1.6	(+)	Sick	77.3
		36	3.2	50	0.5	+	Improved	66.7
6	M	1	2.6	55	1.0	0	Sick	86.9
		6	2.4	53	0.5	(+)	Improved	75.0
		28	4.19	72	0.3	+	Good	82.7
7	M	1	0.99	23	1.3	(+)	Sick	80.7
		14	1.5	27	0.8	+	Good	69.5
8	F	1	2.07	40	2.4	0	Sick	79.5
		22	4.2	60	0.5	+	Good	83.6
9	M	1	4.0	80	0.4	+	Good	80.1
		9	4.0	80	0.5	+	Good	77.0
		28	4.34	84	0.3	+	Good	80.1
10	M	1	1.14	22	2.3	(+)	Sick	80.2
11	F	1	4.7	80	0.4	+	Good	64.3
12	M	1	1.2	29	3.8	0	Sick	69.4
13	M	1	2.3	50	0.2	+	Sick	85.4
14	F	1	3.37	56	0.3	+	Good	74.3
15	M	1	2.85	70	0.7	+	Sick	76.2
Average		..	....	..	...	..		76.6

+ Indicates effective liver extract or liver therapy; 0, indicates no liver or extract given; (+) indicates experimental liver extract which did not contain the effective principle for stimulation of the blood.

The remaining patients who had been treated with liver or liver extract showed a variation of from 59.7 to 93.8 per cent with an average of 76.8 per cent of growth of the root. Care was taken to secure serum from patients of the male sex and from females who were not menstruating.

The coefficient of growth for forty-eight hours was determined, and essentially the same percentages were obtained as those for twenty-four hour periods. Marked toxicity for serum from patients with pernicious anemia was not noted.

As in a given set of seedlings grown from identical seeds under identical conditions, a decided variation in the growth of the root was noted, it was considered advisable to select seedlings which were of approximately the same length for comparative observations. When direct comparisons are made between seedlings of exactly the same original length after a twenty-four hour period, a consistent variation does not seem to exist between those grown in normal serum and those grown in serum from patients with pernicious anemia. In fact, the variation in extremes of growth in blood serum of any type studied does not show any more variation than individual seedlings grown in Shive's solution.

When results obtained so far are considered, one finds that normal blood serum is toxic to seedlings as compared to Shive's solution, but there does not seem to be any evidence that serums of patients with pernicious anemia are any more toxic than is normal blood serum itself. Moreover, there is not any appreciable difference between the coefficients of growth in serums from patients who were not treated and those from patients treated with liver or liver extract.

A further analysis of the records and a comparison of the degree of illness with growth of the roots in centimeter at first glance seems to show a correlation. When the percentage increase in growth in Shive's solutions is calculated, however, only in case 2 is there correlation.

There does not seem to be any relation between the coefficient of growth and clinical condition as measured by the red blood cell count, the hemoglobin percentage and the amount of bilirubin in the blood. We felt that the toxicity of normal blood might be caused by a difference in the hydrogen ion concentration; p_H of a 1 per cent blood solution differs from the p_H of Shive's by 0.4+. When the hydrogen ion value of Shive's solution was changed to correspond to that of the 1 per cent serum solution, by the addition of sodium hydroxide, the growth was equal to and in a few cases greater than that in the normal Shive's solution. It is evident that the slightly higher hydrogen ion value of the serum solutions would tend to enhance the growth, if any difference in growth was evident.

The fact that a 1 per cent serum solution supports more bacterial growth than Shive's solution probably does not explain the difference in growth in the latter solution and in a 1 per cent serum solution, for the bacterial growths at the end of twenty-four hours were slight as indicated by cultures and plates.

The seedlings tested with the serums from patients with secondary anemia (leukemia, infection with *Dibothryocephalus latus*, carcinoma, duodenal ulcer) did not show any marked difference in the rate of

growth from those tested with serum from normal persons or from patients with pernicious anemia (table 2). Their coefficients of growth varied from 60.6 to 88 per cent with an average of 74.7 per cent.

With such slight differences as those found in serums from normal persons and from persons with pernicious anemia, it is impossible for a diagnosis to be based on the percentage increase in growth of seedlings of *Lupinus albus*.

When one surveys this experiment from the standpoint of the practical application of phytopharmacology as a new branch of science,

TABLE 2.—*Serums, Growth of Seedling and Percentage Growth for Twenty-Four and Forty-Eight Hour Periods*

Test Number	Normal with Breakfast	Normal without Breakfast	Anemia, Not Pernicious Anemia	Pernicious Anemia		
				Good Condition	Moderately Sick	Very Sick
1	69.1	....	....	.....	.....	Case 3, 71.2
2	....	....	80.3	Case 9, 80.1	.....	.....
3	87.3	....	....	.....	Case 3, 65.3	Case 1, 67.4
4	97.4	....	....	Case 1, 93.8 Case 3, 84.5	.....	Case 2, 81.0
5	....	....	....	Case 9, 77.0	.....	.....
6	50.0	....	60.6 88.0	.....	.....	.....
7	82.3 82.7	76.9	....	.....	.....	Case 6, 86.9 Case 13, 85.4
8	86.9	85.6	....	Case 2, 85.6	.....	.....
9	81.4	....	....	Case 9, 80.1	Case 6, 75.0	.....
10	....	73.5	....	.....	.....	Case 5, 77.3
11	....	63.4	....	.....	.....	Case 4, 60.1
12	76.2 71.3	88.1	....	Case 2, 93.4		
13	68.4	74.9	75.2			
14	83.7	....	....	Case 1, 73.3 Case 14, 74.3		
15	80.3	....	....	.....	.....	Case 8, 79.5 Case 15, 76.2
16	88.7	....	....	Case 6, 82.7	.....	Case 7, 80.7
17	61.8	....	72.6	Case 4, 75.3		
18	72.4	....	78.3	.....	.....	Case 10, 80.2
19	63.3	....	....	Case 4, 59.7	Case 5, 66.7	
20	74.4	....	....	Case 7, 69.5 Case 11, 64.3		
21	72.9	....	....	Case 8, 83.6		
22	....	....	68.0	.....	.....	Case 12, 69.4
Average	75.2	77	74.7	78.5	69	76.3

several notable points present themselves: Plant growth like animal growth is governed not only by environmental but also by hereditary influences. Thus, one sees that in the foregoing experiments in which environmental factors such as food, temperature, water and humidity are constant, there are marked variations in the growth of the roots of seedlings which must be explained by hereditary factors. It is impossible to estimate these inherited factors of growth from external examination. Even if we select seedlings having cotyledons of the same size and with an equal supply of reserve food, it is conceivable that certain cotyledons contain quantitative chemical differences which may have an important bearing on early growth of the root.

We now control this factor of heredity by using a large number of seedlings and attaching significance to the average. As the science of phytopharmacology progresses, it may be found advisable to secure seeds from a given plant and an inbred strain for comparative observations. Such controls over inherited factors are decidedly favorable in securing accurate results. It is this control that man has over inherited factors in plants, which he does not possess in animals, and the small cost of experiments carried on with plants that makes plants so important to future workers.

SUMMARY AND CONCLUSIONS

1. Seedlings of *Lupinus albus* grown in Shive's nutrient saline solution and containing serum from normal persons showed an average rate of growth of 76 per cent of the rate of growth in Shive's solution alone. The rate of growth was slightly, but not significantly, higher in the serum of persons in the postdigestive state, as compared with those who had taken food a short time before.

2. The growth of seedlings in solutions containing serum from patients with pernicious anemia did not vary significantly from that in serum taken from normal persons.

3. An appreciable difference was not noted between the coefficient of growth for lupine seedlings in serum from patients who were not treated; that in the serum from patients with pernicious anemia who were treated with liver was noted.

4. A relation was not found between the coefficient of growth and the clinical condition of the patients with anemia as measured by degree of illness, red blood cell count, hemoglobin or bilirubin in the blood.

5. With the seedlings used, it was not possible to diagnose pernicious anemia by the effect of blood serum on the growth of the root.

6. Whatever differences of growth may have been influenced by the serums (environmental factors) may have been overshadowed by the inherent variations of growth of the seedlings of different parentage with dissimilar tendencies of rate of growth (hereditary factor). This may account for the differences in the results of the observations from those reported by others.

7. The observation of Macht that serum from patients with secondary anemia does not depress the growth of seedlings is confirmed.

BILE ACIDS IN JAUNDICE*

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The present day evidence, although incomplete, is in favor of the view that the bile acids are formed in the hepatic parenchyma, but what is the evidence on which this assumption is based? For many years, it was assumed that bile pigments were formed in the liver and could not be formed elsewhere. It is becoming increasingly evident that bile pigments are formed largely outside the liver and eliminated through the liver. That bile pigments are formed rapidly and abundantly in the circulation outside the liver is confirmed and extended by observations made by Mann and his collaborators,[1] who employed complete extirpation of the liver and infusion of sugar in experiments lasting from twelve to eighteen hours. Is it possible that bile acids, in like fashion, may be formed outside the liver and merely eliminated by it? A discussion of these points has been made in a recent review [2] and in publications by Whipple and his collaborators,[3] although their explanation that the bile acids are produced in the liver is far from being convincing. Further discussion will be deferred to a later section. In the clinical study of hepatic disease, much interest has centered around the possibilities of dissociated jaundice and variations in bile acid metabolism independent of changes in the bile pigments. Much of this [4] work has been based on variations in the surface tension of the urine, as shown by Hay's test,[5] which is not specific for bile acids and does not permit

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5. Hay, Mathew, 1886. This test was outlined by Müller, H., Jr.: Hay Test for Bile Acids in Urine, Schweiz. med. Wchnschr. **51**:821 (Sept. 8) 1921.

of a quantitative study.[6] The application of van Slyke's gasometric method to the determination of bile acids in small amounts of bile from dogs by Foster and Hooper [7] and by Schmidt and Dart [8] has made possible the accumulation of valuable data concerning the excretion of bile acids in the bile. Schmidt and Merrill [9] have further devised a method of analyzing the bile acid in urine, but these methods are not applicable to the smaller amount of bile acids in the urine and blood.

Szilárd [10] recently described a comparatively simple colorimetric method for the estimation of the bile acids of blood plasma or serum. With this technic, bile acids may be determined in from 4 to 5 cc. of serum. It has been my experience that, within the limits of experimental error, this method yields a quantitative recovery of taurocholic or glycocholic acids when their concentration lies between 10 and 100 mg. per hundred cubic centimeters. The best results are obtained by employing a sufficient volume of plasma or serum so that there will be about 0.5 or 2 mg. of bile acids for the analysis.[11]

Brulé [12] suggested that bile acids are essential for the absorption of fats from the intestine, and it was on this basis that his "hemoconia" test was devised. Normally, there is an increase in ultramicroscopic blood fat after a fat meal; this increase does not occur unless bile acids are present in the duodenum.

A fact established early in the history [13] of the function of bile was the "circulation of the bile acids." There is no evidence, however, for a circulation of the bile pigments.[14] The pigments, apparently, do not fulfill a useful function and are true waste products. Bile acids are rapidly absorbed from the intestinal contents and reappear in the bile. In the dogs in which they created bile fistulas, Foster, Hooper

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and Whipple [15] noted that when moderate amounts of taurocholic acid (about 1.8 Gm.) are given by mouth, about 90 per cent of the contained taurocholic acid is excreted during the first four hours; but when large doses (from 8 to 11 Gm. of taurocholic acid) are administered, the cholagogue effect is prolonged for many hours or days.

In a previous paper,[11] 100 cases of jaundice were reported, and particular attention was paid to the significance of bile acids in the blood. The object of this communication is to report further studies of bile acids in the blood and in the urine in a series of thirty-two cases representing different types of jaundice.

Bernheim,[16] Shattuck, Preston and Browne [17] and Shattuck, Preston and Killian [18] found the icterus index of the blood serum the most dependable gage of the hyperbilirubinemia; consequently, in this investigation the icterus index was utilized for estimation of the bilirubin of the blood serum. My [19] method was employed for the bile acids in the urine, and for the urobilin, Elman and McMaster's [20] method was adopted.

TABLE 1.—*Normal Control Cases*

	Blood				Urine			
	Icterus Index		Bile Acids, Mg. per 100 Cc.		Urobilin, Mg. per 100 Cc.		Bile Acids, Mg. per 100 Cc.	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
10 normal cases...	4.4	7.0	5.0	12.0	0	0	0	Trace
Average.....	5.7		7.0		0		0	

RESULTS

Normal Control Cases.—In table 1 are presented ten normal control cases without suspected or proved injury to the liver. The icterus index was normal, varying from 4.4 to 7. The average figure was 5.7. Van den Bergh's bilirubinemia test was negative in all cases except one, in

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TABLE 2.—*Cholecystitis and Cholelithiasis*

Case	Age	Sex	Date, 1927-28	Blood: Icterus Index	Blood: Van den Bergh Direct	Blood: Van den Bergh Indirect	Blood: Bile Acids, Mg. per 100 Cc.	Urine: 24 Hr. Vol. Cc.	Urine: Urobilin Mg. per 100 Cc.	Urine: Urobilin Mg. per Vol.	Urine: Bile Acids Mg. per 100 Cc.	Urine: Bile Acids Mg. per Vol.	Comment
1. A. A.	49	M	9/26	78.5	++++	++++	30.0						
			9/30	83.3	++++	++++	28.0	.....	...	.....	....	.....	Cholecystitis and cholangeitis
			10/ 6	....	.....	.....	....	270	47	127	8.0	21.6	
			10/ 7	52.8	++++	++++	....	1,000	24	240	11.5	115.0	
			10/ 9	....	.....	.....	....	1,100	3	33	13.6	149.6	(X-ray — pathologic gallbladder; mass density in right upper quadrant)
			10/11	57.5	++++	++++	19.6						
			10/17	44.0	+++	+++	30.0						
			10/20	36.0	+++	+++	32.0	800	94	752	12.0	96.0	
			10/22	51.0	++	+++	26.8						
			10/24	32.6	++	+++	26.8						
			10/30	21.4	±	+	18.5						
2. Y. M.	50	F	9/22	72.0	++++	++++	30.0	740	24	178	13.0	69.2	Acute cholecystitis; cholangeitis (operation)
			9/30	48.4	+++	++++	20.0						
3. E. W.	64	F	10/12	8.3	0	±	28.0	.....	Trace	.....	5.0	.....	Cholecystitis; cholelithiasis (operation)
4. P. F.	34	F	10/ 5	110.2	++++	++++	24.0	2,350	Trace	Trace	11.0	258.5	Cholecystitis; cholelithiasis (operation)
5. M. M.	47	F	11/ 8	19.4	+	++	34.0	1,700	Trace	Trace	15.8	268.6	Cholecystitis; cholelithiasis (operation)
6. D. R.	47	F	11/ 1	7.5	0	±	20.0	320	188	602	3.0	9.6	Cholecystitis; cholelithiasis (operation)
7. H. M.	45	F	11/12	10.0	0	+	30.0	270	6	16	11.8	31.9	Acute ulcerative cholecystitis imposed on a chronic cholecystitis and cholelithiasis (autopsy)
8. C. F.	70	M	2/17	33.3	++	+++	47.5	.....	...	.....	....	.....	Chronic cholecystitis; cholelithiasis, chronic cholangeitis
			2/20	....	.....	.....	....	450	6	27	17.6	79.2	
			2/21	....	.....	.....	....	730	47	343	18.7	136.5	
			2/22	33.3	++	+++	30.0	1,390	188	2,613	15.9	221.0	
			2/23	....	.....	.....	....	670	24	161	13.2	88.4	(Operation)
			2/24	....	.....	.....	....	590	188	1,109	12.8	75.5	(Autopsy)

which the indirect reaction was weakly positive. The bile acids of the blood serum ranged from 5 to 12 mg. per hundred cubic centimeters estimated as sodium glycocholate, the average being 7 mg. Tests for the excretion of urobilin in the urine were negative in all cases. The tests for bile acids in the urine were negative, except in one case that showed a trace.

Cholecystitis and Cholelithiasis.—In table 2 are grouped eight cases of cholecystitis and cholelithiasis, and in seven of these the diagnosis was verified by operation or autopsy. Four patients (patients 1, 2, 4 and 8) were clinically jaundiced, giving figures for the icterus index that were above the zone of latent jaundice. These four cases showed evidence of biliary obstruction. In the remaining instances, the icterus index varied from 7.5 to 19.4. It is significant that in all of these patients there is seen a distinct increase in the concentration of bile acids in the blood serum without any relation to the bilirubin. The concentration of bile acids was greater in some of these cases with low icterus indexes than in those with hyperbilirubinemias. The output of urobilin in the urine varied from traces to 2,613 mg. for twenty-four hour periods. All of these patients excreted bile acids in the urine, ranging from 9.6 to 268.6 mg. for twenty-four hours. It is of interest to note that the bile acids appear in the urine when the concentration of bile acids in the blood serum exceeds 20 mg. per hundred cubic centimeters. There seems to be no relation between the output of bile acids and the excretion of urobilin in the urine.

A. A., a man, aged 49, had a history of clay colored stools for three months. On admission to the hospital, the icterus index was 78.5 and the bile acids, 30 mg. per hundred cubic centimeters of blood serum. A month later, the patient had improved on medical treatment, and the icterus index fell to 21.4 and the bile acids of the blood decreased to 18.5 mg. per hundred cubic centimeters. During this time, the excretion of urobilin for twenty-four hour specimens ranged from 33 to 752 mg., and the output of bile acids in the urine varied from 21.6 to 149.6 mg. for twenty-four hour periods.

Disease of the Liver.—In table 3, data are presented for four patients with diseases of the liver representing varying degrees of hyperbilirubinemia, the icterus index varying from 32.4 to 153. All of these patients presented abnormally high figures for bile acids ranging from 32 to 47.8 mg. per hundred cubic centimeters of serum. Urobilin was excreted in the urine in all cases, except in case 4 (C. P.), that of a patient with carcinoma of the liver associated with complete biliary obstruction. The bile acids were present in the urine in all cases. The amounts, however, were relatively small and fairly constant.

J. S., a girl, aged 9 years, was admitted to the hospital on Sept. 8, 1927, complaining of chronic jaundice with enlargement of the liver and spleen of five months' duration. She had been treated medically without results. Exploratory

TABLE 3.—*Disease of the Liver*

Case	Age	Sex	Date, 1927–28	Blood: Icterus Index	Blood: Van den Bergh Direct	Blood: Van den Bergh Indirect	Blood: Bile Acids, Mg. per 100 Cc.	Urine: 24 Hr. Vol. Cc.	Urine: Urobilin Mg. per 100 Cc.	Urine: Urobilin Mg. per Vol.	Urine: Bile Acids Mg. per 100 Cc.	Urine: Bile Acids Mg. per Vol.	Comment
1. J. S.	8	F	9/ 9	75.0	++++	+++	39.0	...	...	...	....	....	Acute toxic hepatitis (biliary cirrhosis); autopsy
			9/11	....	.....	.....	....	320	6	19	14.6	65.9	
			9/14	78.9	++++	++++	28.6						
			9/15	....	.....	.....	....	500	9	45	13.2	66.0	
			9/21	60.0	++++	++++	25.0						
			9/22	....	.....	.....	....	430	47	202	11.0	47.3	
			9/26	....	.....	.....	....	530	12	64	10.0	53.0	
			9/28	76.5	++++	++++	23.2	290	0	0	7.0	20.3	
			9/29	....	.....	.....	....	...	12	...	7.0		
			10/ 5	....	.....	.....	....	370	Trace	Trace	Trace	Trace	
2. F. M.	53	M	9/ 9	32.4	++	+++	32.0	...	...	...	....	....	Atrophic cirrhosis of the liver; myocarditis; syphilis (autopsy)
			9/14	....	.....	.....	....	370	188	696	7.0	26.0	
3. J. B.	56	M	1/12	122.0	++++	++++	47.8	150	94	141	12.5	18.8	Carcinoma of the liver (autopsy)
			1/16	136.3	++++	++++	36.4	90	24	22	14.1	12.7	
			1/21	112.7	++++	++++	44.5						
4. C.P.	45	F	2/13	153.0	++++	++++	45.5	115	0	0	15.8	18.2	Carcinoma, metastasis to the liver; carcinoma of the esophagus (squamous cell type
			2/14	.....	.....	.....	....	160	0	0	15.0	24.0	
			2/15	.....	.....	.....	....	210	0	0	14.0	29.4	

laparotomy was done and a drainage tube inserted to the gallbladder (October 3), and a section of the liver was removed for microscopic study. The diagnosis was acute toxic hepatitis. Postoperative pneumonia and a marked abdominal distention developed. The patient became progressively worse after the development of pneumonia and died three days after the operation. The icterus index was 75 and the serum bile acids, 39 mg. per hundred cubic centimeters on admission. The bile acids gradually decreased to 23.2 mg. after twenty days, while the bilirubin in the blood remained unchanged. The output of bile acids in the urine for twenty-four hours was 65.9 mg. at first. This gradually decreased to a trace after four weeks and just before death.

Catarrhal Jaundice.—Table 4 presents seven cases of catarrhal jaundice in which the diagnosis was made according to the criteria of Klemperer, Killian and Heyd.[21] A hyperbilirubinemia is seen in all cases and an increase in the bile acids of the blood ranging from 31.2 to 53.5 mg. per hundred cubic centimeters. However, the bile acids in cases 1, 3 and 6 did not show a decrease paralleling the drop in the icterus index; on the contrary, a slight increase was noted in case 1. Case 5 showed a gradual decrease in the bile acids of the blood paralleling the diminution of icterus index. Marked fluctuations were noted in the excretion of urobilin. In some instances (cases 2, 6 and 7), the curve showed peaks of large amounts and then dropped off to a scanty excretion or even a negative reaction within twenty-four hours, with a prompt subsequent rise. All of these patients excreted bile acids in the urine, and marked variations were noted. They ranged from 18.4 to 264.5 mg. for twenty-four hours.

A. O., a boy, aged 7 years, was admitted to the hospital on Dec. 6, 1927, with a history of a sudden onset of jaundice and indigestion. The icterus index was 62.5 and the bile acids in the serum, 31.2 mg. per hundred cubic centimeters; the output of bile acids in the twenty-four hour urine was 93.6 mg. The urobilin test gave a negative reaction, but the following day the urobilin reached 141 mg. for twenty-four hour urine. A week later, the patient had improved on a fat-free diet, rest in bed and magnesium sulphate therapy. The icterus index decreased to 19.5, but the bile acids in the blood remained unchanged. The output of urobilin in the urine increased to 364 for twenty-four hours. The bile acids in the urine decreased to 32 mg. for twenty-four hours.

Obstructive Jaundice.—Five cases of obstructive jaundice have been studied and the data condensed in table 5. An increase in the icterus index was seen in all cases. The bile acids of the blood serum were above normal, but this increase does not parallel the rise in icterus index. Urobilin was absent or was excreted in minute amounts. When the obstruction is relieved, as in case 1, urobilin reappears in the urine.

21. Klemperer, P.; Killian, J. A., and Heyd, C. G.: Pathology of "Icterus Catarrhalis," Arch. Path. **2**:631 (Nov.) 1926. Heyd, C. G.; Killian, J. A., and Klemperer, P.: Pathogenesis of Jaundice, Surg. Gynec. Obst. **44**:489 (April) 1927.

TABLE 4.—*Catarrhal Jaundice*

Case	Age	Sex	Date, 1927-28	Blood: Icterus Index	Blood: Van den Bergh Direct	Blood: Van den Bergh Indirect	Blood: Bile Acids, Mg. per 100 Cc.	Urine: 24 Hr. Vol. Cc.	Urine: Urobilin Mg. per 100 Cc.	Urine: Urobilin Mg. per Vol.	Urine: Bile Acids Mg. per 100 Cc.	Urine: Bile Acids Mg. per Vol.	Comment
1. M. N.	40	F	12/ 9	154.6	++++	++++	....	480	94	451	8.4	40.3	Day specimen
								900	188	1,692	11.8	106.2	Night specimen
			12/12	154.5	++++	++++	32.1						
			12/16	138.8	++++	++++	32.0	.....	...	.....	....	.....	Acute catarrhal jaundice
			12/22	115.4	++++	++++	44.0						
			12/27	.....	.....	.....	....	1,740	188	3,271	15.2	264.5	
2. E. H.	6	F	12/21	29.4	+	++	33.3	340	188	639	15.0	51.0	Acute catarrhal jaundice
			12/27	....	.....	.....	....	260	0	0	11.0	28.6	
3. W. F.	43	F	1/ 5	136.3	++++	++++	45.4	460	188	865	14.5	66.7	Acute catarrhal jaundice
			1/12	120.0	++++	++++	53.5	460	282	1,297	4 0	18.4	with cholecystitis
			1/18	48.4	+++	+++	44.6						
4. E. R.	38	M	10/17	83.3	++++	++++	36.0	.....	752	.....	12.0	....	Acute catarrhal jaundice
			10/19	....	.....	.....	....	.....	376	.....	15.0		
5. P. L.	55	M	10/18	60.0	++++	++++	42.0	1,060	470	4,982	12.0	127.2	Catarrhal jaundice
			10/24	45.5	+++	++++	21.0						
			10/28	32.6	+++	+++	15.5						
6. G. O.	7	M	12/ 7	62.5	+++	++++	31.2	.....	...	.....	....	....	Acute catarrhal jaundice
			12/ 8	....	.....	.....	....	620	0	0	15.1	93.6	Duodenitis
			12/10	....	.....	.....	....	150	94	141	16.5	20.3	
			12/14	19.5	±	+	31.2	260	140	364	12.3	32.0	
7. R. E.	34	M	2/24	49.2	+++	+++	53.5	.....	...	.....	....	.....	Catarrhal jaundice
			2/26	....	.....	.....	....	1,130	94	1,062	17.3	195.5	
			2/27	....	.....	.....	....	580	Trace	Trace	15.0	87.0	
			2/28	44.4	+++	+++	....						

TABLE 5.—*Obstructive Jaundice*

Case	Age	Sex	Date, 1927-28	Blood				Urine					Comment
				Icterus Index	Van den Bergh		Bile Acids, Mg. per 100 Cc.	24 Hr. Vol. Cc.	Urobilin		Bile Acids		
					Direct	Indirect			Mg. per 100 Cc.	Mg. per Vol.	Mg. per 100 Cc.	Mg. per Vol.	
1. J. S.	58	M	10/10	92.5	++++	++++	24.0	1,500	0	0	14.3	214.5	Carcinoma of head of the pancreas; diabetes mellitus
			10/11	....	.....	.....	....	850	0	0	18.0	153.0	
			10/17	157.8	++++	++++	25.0						
			10/18	....	++++	++++	24.0	350	0	0	14.0	49.0	
			10/22	141.4	++++	++++	23.8	900	12	108	15.7	141.3	
			11/ 7	31.5	++	+++	21.0	1,160	Trace	Trace	13.6	157.7	10/24, cholecystogastrostomy
			11/15	23.0	+	±	21.4						
			11/18	....	.....	.....	....	1,100	3	33	11.3	124.3	
			11/19	....	.....	.....	....	600	12	79	Trace	Trace	
			11/21	....	.....	.....	....	920	12	110	0	0	
			11/24	....	.....	.....	....	530	Trace	Trace	0	0	
			11/25	....	.....	.....	....	600	6	36	0	0	
			12/24	....	.....	.....	....	670	12	80	0	0	
2. L. M.	64	F	9/14	107.0	++++	++++	30.0	.....	0	.....	11.2	.....	Obstructive jaundice
			9/18	....	.....	.....	....	.....	0	.....	12.0		
3. A. S.	34	F	11/ 2	50.0	++	+++	34.0	1,800	0	0	13.6	244.8	Carcinoma of head of pancreas (operation)
4. F. W.	71	M	11/14	102.5	++++	++++	14.0	600	0	0	7.0	42.0	Carcinoma of head of pancreas (operation)
5. E. C.	30	F	3/21	16.6	±	+	34.0	.....	...	480	....	.....	Cholemia following chronic cholangeitis with obstruction of common bile duct; operation; death
			3/30	23.0	±	++	35.4	.....	...	230			
			4/12	18.7	0	+	32.8	.....	...	450			
			12/13	49.2	+++	+++	25.8						
			12/15	....	.....	.....	....	1,700	9	153	10.5	178.5	
			1/ 4	35.6	++	+++	20.8	1,500	9	135	9.9	149.5	
			1/ 7	53.5	+++	++++	14.3						

All of these patients excreted bile acids in the urine. The amounts, however, appear to be relatively small and variable, ranging from 42 to 244.8 mg. for twenty-four hours.

Case 1.—J. S., a man, aged 58, with carcinoma of the head of the pancreas, was admitted to the hospital on Oct. 9, 1927. He had had jaundice, clay colored stools and dark urine for about two weeks and sugar in the urine since the middle of August. On October 24, cholecystogastrostomy was done. The postoperative recovery was good. The cholemia and diabetic symptoms were relieved. He was discharged on December 5, with temporary improvement. When he was admitted to the hospital, the icterus index was 92.5 and the bile acids in the serum, 24 mg. per hundred cubic centimeters; urobilin was absent and the bile acids, 214.5 mg. for the twenty-four hour urine. During the following twelve days the icterus index increased to 141.4, but the bile acids of the blood remained unchanged. Two weeks after the operation, the icterus index dropped to 31.5, while the bile acids still remained unchanged. Four weeks after the operation, the test for bile acids in the urine was negative.

Case 5.—E. C., a woman, aged 30, had a history of jaundice of two years' duration. She had been operated on for cholecystectomy on March 12, 1925. A plastic operation on the common duct for stricture was done on March 17, a tube being used. A similar operation was performed on June 11, 1926, and again the patient was readmitted to the hospital on Jan. 2, 1928. On January 5, she was operated on for removal of the tube and reinsertion of a new tube into the common duct. She died two days after the operation. On March 21, 1927, the icterus index was 16.6 and the bile acids in the serum, 34 mg. per hundred cubic centimeters. The excretion of urobilin in the urine was 480 mg. for twenty-four hours. On March 30, the phenoltetrachlorphthalein test was done, and the dye retention in the blood serum after fifteen minutes was 20 per cent, and after sixty minutes, 10 per cent. The icterus index had gradually increased and reached 53.5 before her death, but the bile acids in the blood decreased to 14.3 mg. Output of the bile acids in the twenty-four hour urine was 178.5 mg. on December 15 and 149.5 mg. on January 4.

Cardiac Disease.—In table 6 are presented three cases of cardiac disease. Of these, cases 1 and 3 showed a mild cardiac decompensation; both cases showed an icterus index below 7. Case 2 (E. B.) showed a double mitral valvular disease, with severe cardiac decompensation and an enlarged liver. The icterus index was above 10, and the indirect Van den Bergh test was positive. All of these patients presented high figures for bile acids in the serum, ranging from 22 to 33 mg. per hundred cubic centimeters. Urobilin was found in the urine in all cases, and the bile acids showed a marked increase from 63.6 to 108.5 mg. for twenty-four hours.

Miscellaneous Cases.—In table 7 are presented five miscellaneous cases. Cases 1, 3 and 4 were cases of gastro-enteritis. In case 2 the patient had pernicious anemia and in case 5, typhoid fever.

Case 1.—A. F. gave an icterus index of 12.5, with serum bile acids of 22.2 mg. per hundred cubic centimeters. Urobilin and bile acids were both present in the urine. Inflammation in the duodenum was definitely established.

TABLE 6.—*Cardiac Disease*

Case	Age	Sex	Date, 1927-28	Blood: Icterus Index	Blood: Van den Bergh Direct	Blood: Van den Bergh Indirect	Blood: Bile Acids, Mg. per 100 Cc.	Urine: 24 Hr. Vol. Cc.	Urine: Urobilin Mg. per 100 Cc.	Urine: Urobilin Mg. per Vol.	Urine: Bile Acids Mg. per 100 Cc.	Urine: Bile Acids Mg. per Vol.	Comment
1. C. H.	12	F	12/10	7.0	0	0	33.0						Mitral insufficiency; poliomyelitis
			12/15	....	..	..	....	630	47	293	10.1	63.6	
			12/27	....	..	..	....	510	3	15	9.2	46.9	
2. E. B.	8	F	10/ 2	10.0	0	+	22.0	...	..	...	....	.....	Mitral stenosis and insufficiency; pericarditis; acute endocarditis
			10/19	11.5	0	+	28.6	600	6	36	12.0	72.0	
			10/20	....	..	..	....	850	0	0	11.8	100.3	
			11/ 2	....	..	..	....	750	6	45	11.5	86.3	
			11/16	10.2	0	+	31.2	270	Trace	Trace	8.5	23.0	
3. C. V.	6	M	1/ 8	6.8	0	0	29.8	920	6	55	11.8	108.5	Mitral stenosis and insufficiency; subacute endocarditis; secondary anemia

TABLE 7.—*Miscellaneous Cases*

Case	Age	Sex	Date, 1927-28	Blood: Icterus Index	Blood: Van den Bergh Direct	Blood: Van den Bergh Indirect	Blood: Bile Acids, Mg. per 100 Cc.	Urine: 24 Hr. Vol. Cc.	Urine: Urobilin Mg. per 100 Cc.	Urine: Urobilin Mg. per Vol.	Urine: Bile Acids Mg. per 100 Cc.	Urine: Bile Acids Mg. per Vol.	Comment
1. A. F.	32	M	10/ 8	12.5	±	+	22.2	2,000	12	240	8.5	170.0	Gastro-enteritis
2. S. B.	57	M	11/28	5.4	0	0	16.1	.....	...	...	....	.....	Pernicious anemia; autopsy
			12/ 3	....	..	..	....	750	Trace	Trace	Trace	Trace	
			12/ 4	....	..	..	....	800	0	0	0	0	
			12/ 7	....	..	..	....	560	0	0	0	0	
3. W. S.	41	M	12/11	6.8	0	±	26.5	1,700	0	0	5.5	93.5	Chronic gastritis; perduodenitis
			12/14	....	..	..	....	800	Trace	Trace	6.5	52.0	
4. P. C.	12	F	2/15	4.4	0	0	5.0	900	0	0	0	0	Eczema of skin; chronic enterocolitis
													Typhoid fever
5. B. W.	27	F	12/22	6.8	0	±	8.5	.....	3	...	Trace	.....	High fever
			1/16	7.5	0	±	27.5	600	0	0	4.0	24.0	Crisis

Case 2.—S. B. showed a normal icterus index and slightly increased bile acids in the serum. The urine did not contain urobilin or bile acids.

Case 3.—In W. S., the icterus index was normal, while the bile acids in the blood serum were high, 26.5 mg. per hundred cubic centimeters. Urobilin was not present, but bile acids were found in the urine. This patient had periduodenitis.

Case 4.—P. C. showed low normal figures for the icterus index and bile acids in the serum. Neither urobilin nor bile acids were found in the urine.

Case 5.—At the height of the fever in B. W., the concentration of the bile acids in the blood was normal, 8.5 mg. per hundred cubic centimeters; the icterus index also was normal. Two weeks later, at the crisis, bile acids in the serum increased to 27.5, and the icterus index also showed a corresponding slight increase.

COMMENT

Increased bile acids of the blood serum associated with urinary excretion of bile acids are seen in persons with cholecystitis, disease of the liver, catarrhal jaundice, obstructive jaundice and cardiac decompensation. It is common in patients with cardiac decompensation associated with a mild degree of jaundice and enlargement of the liver.

The presence of bile acids in the blood of persons with obstructive jaundice has been accepted as a fact since the early experiments of Kühne [22] and Huppert.[23] More recently, Rosenthal and Wislicki,[24] Perlzweig and Barron [25] and Snell, Greene and Rowntree [26] obtained positive reactions for bile acids in the blood of patients or animals with obstructive jaundice. Shattuck, Killian and I [11] observed in experiments with animals that phosphorus poisoning produces both a hyperbilirubinemia and an increase in the bile acids of the blood. The increase in bile acids observed after phosphorus poisoning is apparently due to a retention. We also demonstrated that an acute hemolysis increases the bilirubin of the blood serum but does not influence the concentration of bile acids.

Smyth and Whipple [27] reported that choloroform in remarkably small doses incapable of causing recognizable histologic injury to the

22. Kühne, W.: A Study of Jaundice; Physiological Chemical Observation. Arch. f. path. Anat. u. Physiol. **14**:310, 1858.

23. Huppert, H.: The Fate of Bile Acids in Jaundice, Arch. f Heilk. **5**:236, 1864.

24. Rosenthal, F., and Wislicki, L.: Quantitative Determination of Bile Acids in Blood, Arch. f. exper. Path. u. Pharmakol. **117**:8, 1926.

25. Perlzweig, W. A., and Barron, E. G.: New Colorimetric Method for the Determination of Bile Acids in Blood, Proc. Soc. Exper. Biol. & Med. **24**:23 (Dec.) 1926.

26. Snell, A. M.; Greene, C. H., and Rowntree, L. G.: Diseases of the Liver: VII. Further Studies in Experimental Obstructive Jaundice, Arch. Int. Med. **40**:471 (Oct.) 1927.

27. Smyth and Whipple (footnote 3, second reference).

liver or clinical reaction can effect a decrease in the concentration of bile acids in bile from fistulas. In large doses, phosphorus also has a depressant effect on the output of bile acid in bile from fistulas. Moreover, Foster, Hooper and Whipple [28] observed that a functionally deficient liver (Eck fistula) produces less than one-half the normal amount of bile acids during a standard diet period. These authors concluded that such observations present more evidence to substantiate the theory that the bile acids are produced by the liver and not elsewhere in the body. However, it is equally logical to attribute the diminished excretion of the bile acids in the bile to a retention by the liver.

It is of interest to note that the changes in the concentration of bile acids in the blood do not bear any relation to variations in bilirubin. This is seen in tables 2, 6 and 7. Smyth and Whipple found that the injury to the liver produced by liver poisons sometimes depressed, and at other times was without influence on, the excretion of bile pigments. Apparently, then, whatever part the liver plays in bile acid metabolism may in experiments and pathologic conditions be affected independently of its function in bile pigment metabolism. Moreover, it appears possible that the diminished excretion of bile acids in the bile after injury to the liver may be due to a retention and not to a diminished formation.

Acute obstructive or catarrhal jaundice produced a rapid rise in the bilirubin of the blood serum and a marked increase in bile acids associated with urinary excretion of bile acids and bile pigments. In chronic obstructions (table 5), however, the increase in bile acids in the blood is much less, reaching only from three to four times the normal. This smaller concentration of bile acids in the blood in chronic obstructions may be attributed to impairment of the ability of the liver to produce bile acids after long obstructions. However, there is another possible explanation. The production of bile acids in the body is limited. Normally, the bile acids are excreted by way of the bile into the intestine, reabsorbed and again circulated, thus keeping the bile acid in the body at a constant amount. In persons with obstructive jaundice of long standing, however, there is a continuous excretion of bile acids by the kidney, which, of course, cannot be reabsorbed, thus depleting the store of bile acids in the body.

Brakefield and Schmidt [29] produced obstructive joundice in dogs by ligation and subsequent resection of the common bile duct. They noted that the production of the jaundice was followed by a urinary excretion of bile acid and bile pigment, but the output of bile acid decreased and

28. Foster, Hooper and Whipple (footnote 3, first reference).

29. Brakefield, J. L., and Schmidt, C. L. A.: Studies on the Synthesis and Elimination of Certain Bile Components in Obstructive Jaundice, J. Biol. Chem. **67**:523 (Feb.) 1926.

finally became small in quantity. An injection of taurocholic acids led to an increased excretion of this substance. Moreover, it was found that the ability of the jaundiced dog to detoxicate benzoic acid was markedly diminished, and microscopic section of the livers showed extensive morphologic changes. According to the observations of these authors, the diminished excretion of bile acids was due to an impairment of the ability of the liver to produce the bile acids. J. S. (case 1, table 3) and J. S. (case 1, table 5) had high bile acids in the blood and urinary excretion of bile acids. The concentration of bile acids in the blood decreased on subsequent examinations but did not approach the normal level, although the urinary excretion of bile acids gradually diminished and finally disappeared. In E. C. (case 5, table 5), who had long standing obstructive jaundice, the concentration of bile acids in the blood was 34 mg. per hundred cubic centimeters. Ten months later, the amount of bile acids in the blood was still 20.8 mg. per hundred cubic centimeters above the normal, while the output of bile acids in the urine was 149.5 mg. for twenty-four hours. These facts cannot be explained by the theory of Brakefield and Schmidt.

In persons with catarrhal jaundice (table 4) are seen marked variations in the excretion of urobilin, in some instances ranging from a negative reaction to more than 1 Gm. in twenty-four hours. Wallace and Diamond [30] and Shattuck, Killian and I [11] reported great variations in excretion of urobilin in persons with catarrhal jaundice, the curve showing peaks of large amount and dropping off to a scanty excretion or a negative reaction within twenty-four hours, with a prompt subsequent rise. However, the urobilin never remains permanently absent from the urine in persons with catarrhal jaundice. In obstructive jaundice (table 5), the urobilin was absent or was excreted in minute amounts. When the obstruction is relieved, urobilin reappears in the urine. If the excretion of urobilin in the urine is to be utilized as an aid in the differential diagnosis between obstructive and catarrhal jaundice, the determinations of urobilin in the urine must be continued over a period of not less than five days.

SUMMARY

The bile acids of normal blood serum varied from 5 to 12 mg. per hundred cubic centimeters, estimated as sodium glycocholate. The average was 7 mg. per hundred cubic centimeters.

The urine of normal subjects showed a negative reaction for bile acids.

30. Wallace, A. B., and Diamond, J. S.: The Significance of Urobilin in the Urine as a Test for Liver Function with a Description of a Simple Quantitative Method for Its Estimation, Arch. Int. Med. **35**:698 (June) 1925.

Patients with cholecystitis, disease of the liver, catarrhal jaundice, obstructive jaundice, cardiac decompensation and duodenitis showed a marked increase in the bile acids of the blood serum, accompanied by urinary excretion of bile acids. The amount of bile acids in the urine, however, appears to be relatively small and extremely variable.

The changes in the concentration of bile acids in the blood do not bear any relation to variation in bilirubin of the blood.

The bile acids appear in the urine when the concentration of bile acids in the blood serum exceeds 20 mg. per hundred cubic centimeters.

Acute obstructive or catarrhal jaundice produced a rapid rise in the bile acids of the blood serum from about five to seven times the normal figure. In chronic obstructions, however, the increase in bile acids in the blood is much less, to only three or four times the normal. This smaller concentration of bile acids in the blood in chronic obstructions may be explained thus:

The production of bile acids in the body is limited. Normally, they are excreted by way of the bile into the intestine; then they are reabsorbed and again circulated, thus keeping the bile acids in the body at a constant amount. In obstructive jaundice of long standing, however, there is a continuous excretion of bile acids by the kidney, which, of course, cannot be reabsorbed, thus depleting the store of bile acids in the body.

DISTRIBUTION OF BLOOD SUGAR BETWEEN CORPUSCLES AND PLASMA IN DIABETIC AND IN ALIMENTARY HYPERGLYCEMIA*

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It has been demonstrated that the generally accepted view of an equal or nearly equal distribution of sugar between corpuscles and plasma in human blood is erroneous.[1] The evidence was based on true sugar values, obtained as the difference between apparent sugar (total reduction) and nonsugar-reducing substances.

It has long been known that nonfermentable substances constitute a part of the sugar values estimated by the usual reduction methods, but—owing to reasons set forth elsewhere[2]—these substances eluded quantitative determination to such an extent that some investigators asserted that almost half of the reduction of protein-free filtrates of normal blood is due to nonfermentable constituents, while others denied altogether the existence of such substances. A simple procedure devised by me, involving fermentation by means of thoroughly washed yeast, permits the rapid and complete removal of fermentable sugars from blood and other biologic fluids, and thus the accurate determination of nonfermentable reducing substances or "residual reduction."

Two interesting observations have resulted from the estimation by this method of the reducing nonsugars in human blood; one, the remarkable constancy of the amount, and the other, the unequal distribution of these substances between corpuscles and plasma. The average quantity of reducing nonsugars is 40 mg. per cent in corpuscles, while it is only 8 mg. per cent in plasma. From this it follows that the ratio, $\frac{\text{corpuscle sugar}}{\text{plasma sugar}}$, a convenient expression for the distribution of sugar, is grossly distorted by the reducing nonsugar fractions included in the apparent sugar values. This ratio, as expressed by former investigators, was $\frac{\text{apparent corpuscle sugar}}{\text{apparent plasma sugar}}$, whereas the correct formulation is $\frac{\text{true corpuscle sugar}}{\text{true plasma sugar}} = \frac{\text{apparent corpuscle sugar}-40}{\text{apparent plasma sugar}-8}$.

The fallacy arising from the use of apparent sugar values is brought out in a previously published report of the observations on thirty-six

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* From the Laboratory of the Jewish Hospital of St. Louis.

1. Somogyi, Michael: The Distribution of Sugar in Normal Human Blood, J. Biol. Chem. **78**:117, 1928.

2. Somogyi, Michael: Reducing Nonsugars and True Sugar in Human Blood, J. Biol. Chem. **75**:33, 1927.

specimens of normal human blood,[1] which yielded the following results:

	Lowest	Highest	Average
True distribution ratio.....	0.66	0.95	0.77
Apparent distribution ratio.....	0.96	1.35	1.10

According to the apparent sugar values 100 cc. of corpuscles contain, on an average, 1.1 mg. of sugar for each milligram in 100 cc. of plasma, in conformity with the accepted view of a nearly equal distribution. The true sugar values, however, show a much lower relative sugar content in the corpuscles, namely 0.77 mg. for each milligram in an equal volume of plasma. The error in the distribution ratio, owing to the acceptance of apparent sugar as true sugar, is $\frac{(1.10-0.77)100}{0.77}=43$ per cent.

TABLE 1.—*Influence of the Blood Sugar Level on the Discrepancies Between the Apparent and True Distribution Ratios*

True Sugar, Mg. per 100 Cc. of			True Ratio, Corpuscle Sugar to Plasma Sugar	Apparent Sugar,* Mg. per 100 Cc. of			Apparent Ratio, Corpuscle Sugar to Plasma Sugar
Blood	Corpuscles	Plasma		Blood	Corpuscles	Plasma	
9	7.5	10		33	47.5	18	2.64
17.5	15	20		41.5	55	28	1.96
35	30	40	0.75	59	70	48	1.46
84	72	96		108	112	104	1.08
175	150	200		199	190	208	0.92
263	225	300		287	265	308	0.86
350	300	400		374	340	408	0.84
438	375	500		462	415	508	0.82

* Apparent blood sugar = true blood sugar + 24; apparent corpuscle sugar = true corpuscle sugar + 40; apparent plasma sugar = true plasma sugar + 8.

The discrepancy between the apparent and true ratio is variable, its magnitude depending mainly on the concentration of sugar in blood. In order to illustrate this relationship a possible series of specimens of blood will be considered in which the true distribution ratio is uniformly 0.75 and the corpuscle volume is 50 per cent; the reducing nonsugars are represented by the average values of 40 and 8 mg. per cent in the corpuscles and in the plasma, respectively, while the blood sugar varies from extremely low to extremely high concentrations. Table 1 shows the extent of the disguising effect of the reducing nonsugars on the distribution ratio at various blood sugar levels. The same is shown graphically in chart 1, wherein the curves were constructed by plotting distribution ratios against blood sugar values.

It is evident that the lower the blood sugar concentration the more pronounced is the discrepancy between the two ratios, that is, the more misleading becomes the apparent ratio. Especially changes in the direc-

tion of hypoglycemia entail a sudden rise in the apparent ratio. At a normal fasting sugar level of 108 mg. per hundred cubic centimeters a nearly equal distribution is found, but as the blood sugar goes down to 59 mg. per hundred cubic centimeters, the apparent ratio indicates 1.46 per hundred cubic centimeters of corpuscle sugar for 1 mg. per hundred cubic centimeters of plasma sugar, and at 33 mg. per hundred cubic centimeters of blood sugar, the apparent ratio rises to 2.64, whereas the true ratio is identical at all of these blood sugar levels. Ultimately, as the blood sugar progressively diminishes, the apparent ratio approaches the maximum value of 5:1, a limit reached only when the true sugar becomes zero, and the entire

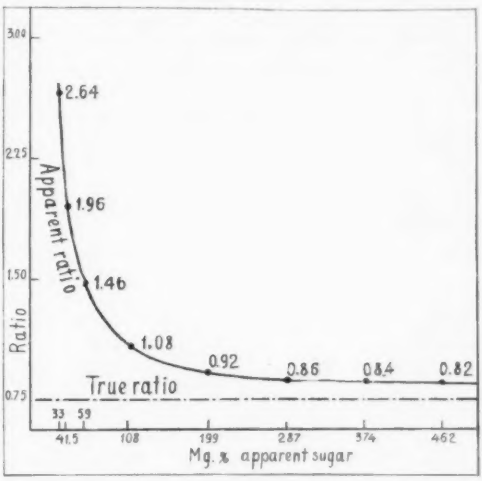

Chart 1.—The ratio expressing the distribution of blood sugar between corpuscles and plasma is distorted by the reducing nonsugars (residual reduction), especially at lower blood sugar levels.

reduction is due to reducing nonsugars. On the other hand, as the blood sugar rises to hyperglycemic levels, the divergence between the apparent and the true ratio gradually decreases, and the two values asymptotically approach identity.

As can be seen, the apparent ratio simulates changes in the distribution of the blood sugar, when actually there are none. As previous investigators have based some interesting conclusions concerning the distribution of blood sugar under various conditions on apparent sugar values, I considered it desirable to study such questions in the light of true sugar values.

John [3] and Wiechmann [4] observed a nearly equal distribution of the sugar between corpuscles and plasma in the blood of fasting normal persons, but in the blood of diabetic patients they found a higher sugar content in the plasma than in the corpuscles. Both authors believe that some constituent of the diabetic plasma inhibits the permeability of the erythrocytes for dextrose. Loewi [5] accepts this view, and claims to have demonstrated in the plasma of diabetic patients the existence of a hormone (glycemin) which lowers the "sugar binding or sugar fixing ability" of the erythrocytes.

TABLE 2.—*Distribution of Sugar in Blood of Diabetic Patients*

Number	Apparent Sugar, Mg. in 100 Cc. of		Reducing Nonsugars, Mg. in 100 Cc. of		True Sugar, Mg. in 100 Cc. of		Ratio, Corpuscle Sugar / Serum Sugar	
	Corpuscles	Serum	Corpuscles	Serum	Corpuscles	Serum	For Apparent Sugar	For True Sugar
1	231	259	43	8	188	251	0.90	0.75
2	161	179	38	8	123	171	0.90	0.70
3	174	195	44	8	130	187	0.90	0.70
4*	215	231	51	10	164	221	0.93	0.74
5	148	159	38	9	110	150	0.93	0.73
6	220	229	39	8	181	221	0.96	0.82
7	169	162	43	7	126	155	1.04	0.81
8	191	191	44	13	147	178	1.00	0.83
9	208	225	37	8	171	217	0.92	0.79
10	243	267	44	14	199	253	0.99	0.79
11	200	227	35	8	165	219	0.88	0.75
12‡	287	302	42	8	245	294	0.95	0.83
13	140	150	38	8	102	142	0.93	0.72
14	137	137	38	8	99	129	1.00	0.77
15	144	142	40	8	104	134	1.01	0.77
16	158	166	37	9	121	155	0.95	0.78
17‡	251	290	39	8	212	282	0.90	0.75
Lowest							0.85	0.70
Highest							1.04	0.83
Average							0.95	0.77

* Specimen of blood withdrawn thirty minutes after the ingestion of 100 g. of dextrose.
‡ Specimen of blood withdrawn two hours after the ingestion of 100 g. of dextrose. The other specimens were obtained before breakfast.

In a series of experiments in which I examined the blood of seventeen diabetic patients, I obtained results—given in table 2—that fully bear out the view of John and of Wiechmann, as far as the apparent sugar values are concerned. The average apparent ratio is 0.95 as against 1.10 in normal blood, indicating that the relative sugar content of the corpuscles is 15 per cent lower in blood from diabetic patients than in

3. John, H. J.: Distribution of Sugar in Whole Blood, Plasma and Corpuscles; Permeability of Red Corpuscles for Sugar in Diabetic and Nondiabetic Cases, Arch. Int. Med. **31**:555 (April) 1923.

4. Wiechmann, E.: Zur Frage der Permeabilität der roten Blutkörperchen für Traubenzucker unter besonderer Berücksichtigung des Diabetes, Ztschr. f. d. ges. exper. Med. **41**:462, 1924.

5. Loewi, O.: Glykämin und Insulin, Klin. Wchnschr. **6**:2169, 2173, 2175, 1927.

that from normal subjects. The true sugar values, however, disclose an average distribution ratio of 0.77, the same as was found in normal blood.

In an endeavor to obtain the most conclusive results possible, I chose for my experiments only patients with more or less severe diabetes. In five cases, sugar tolerance tests were performed by following the changes in the blood sugar level during three hours after the administration of 100 Gm. of dextrose. These tests, presented in table 3, fairly characterize the condition of these patients; at the same time, they demonstrate the absence of any connection between the severity of the diabetes and the distribution of the blood sugar. In case 1, the patient, a "total" diabetic, had a distribution ratio of 0.75, the same as in case 17, the mildest of the five cases, while in case 6, with a tolerance curve closely resembling that in case 1, the high ratio of 0.83 was found. These were variations not in the least different from those occurring in normal

TABLE 3.—*Sugar Tolerance Tests*

Time After Ingestion of 100 g. of Dextrose	No. 1	No. 4	No. 6	No. 12	No. 17
	Mg. Sugar in 100 Cc. of Blood				
0 (fasting)	265	151	240	145	140
30 minutes	385	230	405	225	233
60 minutes	459	313	440	275	324
120 minutes	484	356	504	295	272
180 minutes	472	353	471	247	194
Distribution ratio	0.75	0.74	0.82	0.83	0.75

specimens, and it is safe to conclude that diabetes mellitus does not have any effect on the distribution of sugar between corpuscles and plasma; in other words, it does not influence the ability of the corpuscles to take up (fix, bind) dextrose.

Careful inspection of the experimental data of the authors just quoted leads one to surmise that their unawareness as to the rôle of the reducing nonsugars is not the exclusive cause responsible for the conflict between their results and those reported in this article. Wiechmann, for instance, found 13 and 15 mg. per hundred cubic centimeters of apparent sugar in the blood corpuscles of a healthy man. As the reducing nonsugars in corpuscles alone yield considerably higher values by the method of Folin and Wu—the one employed by Wiechmann—such low values are obviously due to some error in analytic technic.

The distribution of blood sugar in hyperglycemic conditions of nondiabetic genesis has been studied by several workers. In 1912, Höber [6] reported that in alimentary and in epinephrine hyperglycemia

6. Höber, R.: Ueber die Verteilung des Blutzuckers auf Körperchen und Plasma, Biochem. Ztschr. **45**:206, 1912.

the increment in the blood sugar goes eminently to the plasma, that is, the relative sugar concentration in the corpuscles is lowered. Tachau,[7] Wishart,[8] Folin and Berglund[9] and Rona and Sperling[10] found a relatively greater increase in the concentration of the plasma sugar during alimentary hyperglycemia. Loewi[5] said that he believed he had shown the opposite of this, namely, a substantial and progressive increase in the "sugar fixing ability" (relative sugar content) of the corpuscles during three hours following the administration of sugar by mouth.

TABLE 4.—*Showing that Changes in the Level of the Blood Sugar do not Affect the Distribution Ratio*

No.	Case	Apparent Sugar, Mg. in 100 Cc. of		True Sugar, Mg. in 100 Cc. of		Ratio, Corpuscle Sugar / Serum Sugar	
		Corpuscles	Serum	Corpuscles	Serum	For Apparent Sugar	For True Sugar
1	Normal man, fasting	112	99	76	90	1.13	0.84
	30 minutes after dextrose	184	184	148	175	1.00	0.85
	180 minutes after dextrose	77	59	41	50	1.31	0.82
	210 minutes after dextrose	83	66	49	58	1.26	0.84
2	Normal man, fasting	105	101	68	94	1.04	0.72
	30 minutes after dextrose	130	129	98	122	1.00	0.77
	210 minutes after dextrose	87	73	50	66	1.19	0.76
3	Normal man, fasting	111	89	75	81	1.25	0.93
	30 minutes after dextrose	178	167	142	159	1.07	0.90
	180 minutes after dextrose	93	74	57	66	1.26	0.86
4	Normal man, fasting	110	103	72	93	1.07	0.77
	180 minutes after dextrose	121	110	83	100	1.10	0.83
5	Normal woman, fasting	124	128	84	120	0.98	0.70
	30 minutes after dextrose	181	205	141	197	0.89	0.72
6	Normal man, fasting	108	110	69	101	0.98	0.68
	60 minutes after dextrose	150	161	109	154	0.93	0.71
7	Diabetic, fasting	140	150	102	142	0.93	0.72
	180 minutes after dextrose	304	358	266	350	0.85	0.76
8	Diabetic, fasting	137	137	90	129	1.00	0.77
	60 minutes after dextrose	230	265	192	257	0.87	0.75

My observations concerning the conditions in alimentary hyperglycemia, and the ensuing hypoglycemia—which is thought to be essentially identical with insulin hypoglycemia—are recorded in table 4. These experiments were carried out on six healthy and two diabetic subjects. The former received 120, the latter 90 Gm. of dextrose by mouth after blood had been withdrawn during the fasting condition.

7. Tachau, H.: Ueber die Verteilung des Blutzuckers auf Blutkörperchen und Plasma, Ztschr. f. klin. Med. **79**:421, 1914.

8. Wishart, M. B.: The Permeability of Blood Corpuscles to Sugar, J. Biol. Chem. **44**:563, 1920.

9. Folin, O., and Berglund, H.: Some New Observations and Interpretations with Reference to Transportation, Retention and Excretion of Carbohydrates, J. Biol. Chem. **51**:213, 1922.

10. Rona, P., and Sperling, M.: Ueber die Verteilung der Glucose auf Plasma und Blutkörperchen, Biochem. Ztschr. **175**:253, 1926.

Further specimens of blood were obtained at intervals after the ingestion of the sugar. The results require little comment. The apparent sugar values simulate changes in the distribution of the blood sugar, which are in accord with the conclusions of previous workers. Experiment one in table 4—graphically represented in chart 2—is an apt example to illustrate this: When, during the absorption of dextrose, the blood sugar rises to a hyperglycemic level, the concentration of the plasma sugar seemingly increases more rapidly than that of the corpuscle sugar, and the apparent ratio indicates a drop of 13 per cent in the relative sugar content of the corpuscles. Again, as the blood later recedes to a

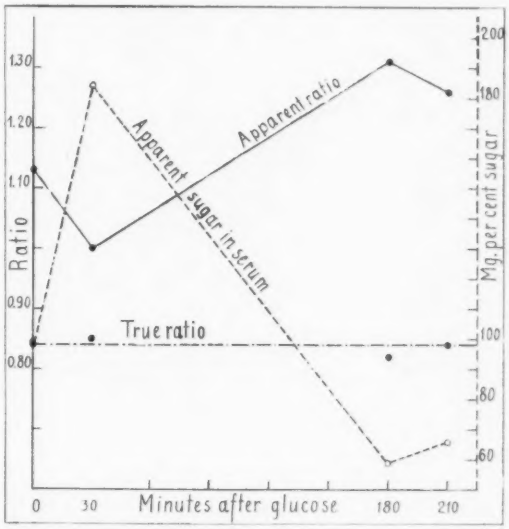

Chart 2.—True sugar values show no alteration in the distribution of blood sugar during alimentary hyperglycemia, while apparent sugar values simulate substantial changes.

hypoglycemic level, the plasma appears to lose of its sugar content faster than the corpuscles, and—as expressed by the apparent ratio—the relative concentration of the corpuscle sugar is increased by 31 per cent. According to the evidence of the true sugar values, however, the ratio of distribution remains largely unchanged throughout the rise and fall of the blood sugar concentration in all of the cases given in table 4, regardless of the normal or diabetic condition.

According to Rona and Sperling,[10] during the decline of the blood sugar concentration subsequent to alimentary hyperglycemia, the corpuscles lose sugar more rapidly than the plasma, often showing an

abruptly steep drop. My observations are contrary to this even in regard to apparent sugar values. Rona and Sperling derived their conclusion from two experiments on a single human subject, and chiefly from observations on rabbits that were given perorally 15 Gm., in one case 30 Gm., of dextrose dissolved in from 50 to 100 cc. of water. Their results are so inconsistent and conflicting among themselves that an interpretation of their data in any direction appears to be more or less arbitrary.

Loewi's work [5] concerning alimentary hyperglycemia is at a decided variance to the results of all the investigators quoted as well as to my own. Loewi claims that the relative sugar concentration of the corpuscles (sugar-fixing ability) in dogs and rabbits rises and declines in a remarkable parallelism with the concentration of the blood sugar. In nondiabetic human subjects, too, the concentration of the corpuscle sugar rises more rapidly than that of the plasma sugar, but during the decline of the blood sugar level the corpuscles give up their sugar content so much more slowly than the plasma that the relative sugar concentration in the corpuscles is at a maximum three hours after the peroral administration of dextrose, when the concentration of the blood sugar had already receded to its lowest level. My observations as shown in chart 2, are in a diagonal contrast to those of Loewi. The curve of the apparent distribution ratio, instead of being parallel to the curve of the plasma sugar (which largely follows the course of the blood sugar), is more of a mirror image to it. In a subsequent paper, dealing with the influence of insulin on the distribution of blood sugar, I shall revert to the discussion of Loewi's observations, which he correlates with effects of endogenous insulin.

SUMMARY

1. The distribution of blood sugar between corpuscles and plasma can be determined only from true sugar values. The substitution of apparent sugar for true sugar leads to erroneous conclusions.

2. The relative sugar content of corpuscles is the same in blood of diabetic and nondiabetic subjects; thus, the conclusion that the sugar fixation by corpuscles is inhibited in the blood of diabetic patients (Wiechmann, Loewi) is erroneous.

3. The distribution of the sugar is unaffected by changes in the blood sugar level caused by alimentary factors.

THE EFFECT OF PARATHYROID EXTRACT ON CERTAIN FACTORS UNDERLYING THE PRODUCTION OF EDEMA*

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It is generally conceded that in several types of edema there is some disturbance in the inorganic ion balance of the body. This is particularly true of edema associated with various forms of renal disease. Whether this imbalance as evidenced by retention of salts, particularly sodium chloride, within the tissues, plays a primary or a secondary rôle is a subject much disputed. The difficulty, if not impossibility, of reproducing edema experimentally has delayed the solution of this problem.

Experimental studies indicate that, apart from the comparatively simple form due to lymphatic obstruction, the production of edema is dependent on several factors, the individual importance of which varies in different instances. The chief of these may be enumerated as follows: (1) increased capillary permeability; (2) alterations in osmotic pressure, and (3) increased affinity of tissue colloids for water. The exact relation of the retention of salt to these phenomena and to the accumulation of fluid in the tissues has been the subject of prolonged investigation.

Edema may be classified as inflammatory and noninflammatory and may be considered under these headings.

RESULTS OF PREVIOUS INVESTIGATIONS

Under normal conditions, the wall of the capillary acts as a semipermeable membrane, allowing free diffusion of water and certain crystalloids, such as urea, and a limited diffusion of other crystalloids. It is impermeable to most colloids. It has been demonstrated, however, that normally a variation in permeability exists in different organs. Starling [1] showed that whereas the lymph flowing from the lower extremities had a protein content of from 2 to 4 per cent, that from the liver contained from 6 to 8 per cent. The work of Krogh [2] has thrown considerable light on this subject. He has shown [3] that a definite

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1. Starling, E. H.: The Physiological Factors Involved in the Causation of Dropsy, Lancet **1**:1267 (May 9) 1896.

2. Krogh, A.: The Anatomy and Physiology of Capillaries, New Haven, Yale University Press, 1922.

3. Krogh, A., and Harrop, G. A.: Some Observations on Stasis and Edema, J. Physiol. **54**:125, 1920-1921.

sequential relationship exists between stimulation, dilatation and increased permeability of the capillaries, and that every muscular effort results in dilatation of the capillaries. Hence, there is a constant alteration in permeability in response to the needs of the tissue, probably dependent on the local accumulation of carbon dioxide, lactic acid and other metabolic products.

The high protein content of inflammatory exudates is an indication of an increase in the permeability of the walls of the capillaries. Oswald [4] stated, in evidence of this fact, that the walls of the capillaries of inflamed areas are permeable, not only to albumin and pseudoglobulin, but also to euglobulin and fibrinogen, the viscosity of which is relatively high. The factor of permeability, however, although of great importance, is not the only one involved. The accumulation of products of the disintegration of tissue at the site of inflammation may result in dilatation of the capillaries and in increased permeability, as proposed by Krogh. In addition, Schade [5] has demonstrated in these areas the development of an extremely high osmotic pressure and an increase in hydrogen ion concentration. Either of these conditions may be responsible for the local accumulation of fluid, the former through the operation of the forces of osmosis, the latter through the increase in the capacity for hydration of the tissue colloids accomplished by the increased acidity. The latter factor, the significance of which was developed chiefly by Fischer,[6] will be considered later.

The influence of inorganic ions, particularly sodium, potassium and calcium, on the permeability of the membranes of the cells is well known, normal permeability being dependent to a considerable degree on the presence of a normal balance between the inorganic ions in the fluid which bathes the cell. Calcium definitely diminishes the permeability of the cell. In fact, Loeb [7] attributes the general inhibiting action of the calcium ion to this diminution in permeability. Osterhout [8] believed that sodium and calcium combine with some constituent of the cell, and that the permeability varies according to the relative proportion in which these two combinations of the constituents of the cell are present.

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4. Oswald, A.: Ueber den Chemismus der Entzundung, Ztschr. f. exper. Path. u. Therap. **8**:226, 1910.

5. Schade, H.: Die Molekular-pathologie in ihren Verhaltnis zur zellular Pathologie und zum klinischen Krankheitzbild am Beispiel der Entzundung, München. med. Wchnschr. **71**:1, 1924.

6. Fischer, M. H.: Oedema and Nephritis, ed. 3, New York, John Wiley & Sons, 1921.

7. Loeb, J.: The Influence of Electrolytes on the Electrification and Rate of Diffusion of Water through Collodion Membranes, J. General Physiol. **1**:717, 1918-1919.

8. Osterhout, W. J. V.: Antagonism and Permeability, Science **45**:97, 1917.

Hamburger [9] stated that the relation of calcium, sodium and potassium ions in a perfusing fluid is the determining factor in the production of edema; the calcium ions tend to make the capillaries impermeable, at the same time causing their constriction.

Investigation of the effect of treatment with calcium on experimentally produced inflammatory edema has yielded somewhat contradictory results. Januschke [10] found that the typical inflammatory reaction produced by mustard oil was prevented by the previous administration of calcium salts. Rosenow [11] found that calcium and epinephrine acted in a similar manner in inhibiting the formation of inflammatory exudates. Negative results have been reported by other investigators, and it is probable, as suggested by Loeb,[12] that the difference in amount and proportion of calcium employed may account for the variable results obtained. In general, the experimental observations seem to indicate that calcium is much more effective in preventing the full development of inflammatory edema than in influencing an already existing process.

Calcium chloride in large doses has been used clinically in the treatment of patients with acute inflammatory conditions, particularly serofibrinous pleurisy. Oriani [13] and Thoné [14] reported good results, with subsidence of the fever and resorption of the effusion. According to Blum [15] and others, calcium chloride diminishes the sodium of the blood and prevents it from migrating from the blood into the inflammatory area. As the current of water follows sodium, the accumulation of fluid ordinarily accompanying the inflammatory process is inhibited. However, it would appear probable that whatever effect calcium has on this type of edema, it is exerted largely through its influence on the permeability of the cell.

NONINFLAMMATORY EDEMA

When one turns to the consideration of noninflammatory edema, as typified by that accompanying cardiac and renal disease, an apparently different problem is encountered. The fact that the fluid of edema in

9. Hamburger, R. J.: Ueber die bedeutung der Kalium und Calciumionen für das kunstliche Oedem und für die Gefässweite, Biochem. Ztschr. **129**:153, 1922.

10. Januschke, H.: Ueber Entzundungshemmung, Wein, klin. Wchnschr. **24**:869, 1913.

11. Rosenow, G.: Der Einfluss parenteraler Calcium zufuhr auf die Durchlässigkeit der Gefässwand, Ztschr. f. d. ges. exper. Med. **4**:427, 1916.

12. Loeb, L.: Edema, Medicine **2**:190, 1923.

13. Oriani, F.: Calcium Chloride in Treatment of Effusions, Clin. med. ital. **56**:475 (Dec.) 1925.

14. Thoné: Calcium Chloride in Exudative Pleurisy, Arch. méd. belges. **78**:1 (Jan.) 1925.

15. Blum, L; Aubel, E., and Hausknecht, R.: Action diuretique des sels de calcium: mechanisme de cette action, Compt. rend. Soc. de biol. **85**:950, 1921.

these conditions is relatively low in protein indicates that increased permeability of the capillaries probably plays a relatively minor part in its causation. However, there is considerable evidence to suggest that this factor is involved to some degree. The work of Morawitz and Denecke [16] suggests that in glomerular nephritis, typhoid, scarlet fever, purpura and acute endocarditis there is a state of increased permeability of the capillaries. They assumed this to be due to toxic injury of the vessels. However, in cardiac edema they found the permeability to be normal. Recently, Skelton [17] found that following hemorrhage various tissues give up water to the blood stream, in proportion to the normal variation in the permeability of the capillaries; this was demonstrated in different organs by Starling; [1] thus, the liver lost the greatest amount of water per unit of mass, the skin and intestines a lesser amount and the muscles the least amount.

Cohnheim [18] believed that in nephritis the vascular injury with resultant increase in permeability is secondary to retention of toxic substances in the blood stream, but Senator [19] considered that the vascular and renal injury are due to the same cause. Wells [20] said that it is probably better to consider the toxic effect as applying to all cells rather than merely to capillaries. Apparently, the edema cannot be explained by the mere diminution of the functional area of the kidney in nephritis, for neither bilateral nephrectomy nor ureteral occlusion necessarily results in accumulation of fluid within the tissues. Neither can cardiac edema be satisfactorily demonstrated as being due entirely to increase in venous pressure dependent on myocardial failure. The great bulk of accumulated evidence seems to indicate that the mechanism of osmosis and altered affinity of the tissue colloids for water are the factors chiefly concerned in these types of edema.

The importance of the retention of salt in the causation of edema was emphasized particularly by Widal.[21] Although many investigators believe this retention to be the primary factor, others consider it secondary to the accumulation of fluid in the tissues. Fischer [6] held that the primary factor is an increase in the acidity of the tissue, which causes an increased affinity of the tissue colloids for water, with resulting

16. Morawitz, R., and Denecke, G.: Ueber Quellungs-vorgänge in subcutanen Gewebe, Biochem. Ztschr. **127**:47, 1922.

17. Skelton, Harold: The Storage of Water by Various Tissues of the Body, Arch. Int. Med. **40**:140 (Aug.) 1927.

18. Cohnheim, J., and Lichtheim, L.: Ueber Hydrämie und hydrämisches Oedem, Virchows Arch. f. path. Anat. **69**:106, 1877.

19. Senator, H.: Ueber die Wassersucht bie Nierenkrankheiten, Klin. Wchnschr. **33**:165, 1895.

20. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925, p. 387.

21. Widal, F.: La cure de dechlorination, Bull. et mém. Soc. d. hôp. de Paris **20**:773, 1903.

retention of salts, particularly sodium chloride. He stated that the proportion of water present in any cell or fluid containing colloids is determined by the character of the colloids, the reaction of the solution and the nature of its electrolyte content. The fact that the retention of salt often occurs in fevers without edema would suggest that some additional condition is necessary. The work of Cohnheim and Lichtheim,[18] and of Müller [22] and many others indicates that increased permeability of the capillaries may be an essential factor.

Considerable light has been thrown on certain phases of this problem by McClure and Aldrich.[23] They found that a small amount of physiologic solution of sodium chloride injected intradermally is entirely taken up by normal tissues in from sixty to eighty minutes. In tissues which are the site of edema or in which a tendency to edema is present, as in nephritis and cardiac disease, the injected fluid disappears more rapidly. This fact is interpreted as indicating an increase in the affinity of the tissues for water, supporting Fischer's contention that variations in the capacity of hydration of tissue colloids play a major rôle in the production of cardiac and renal edema.

The exact mechanism of the action of the various cations in this connection is not clearly understood. However, certain facts have been critically demonstrated. In many cases of edema, particularly those occurring in association with nephritis, there is retention of sodium within the tissues. Whether this retention is primary or secondary to the accumulation of fluid is an open question. However, there cannot be any doubt that it is an essential factor, for in a large proportion of such cases restriction of the intake of sodium is followed by increased elimination of this element chiefly by the kidneys, a greatly increased fluid output and a diminution in the edema.

Naturally, as a result of the known antagonistic actions of sodium and calcium in the body, the effect of the latter on the process of edema has been studied extensively. In general, it has been found, although some contradictory results are reported, that the administration of calcium salts in large doses produces an effect on edema comparable to that obtained by the restriction of the intake of sodium.

Maase and Zondek [24] reported extremely favorable results from the use of calcium therapy in the peculiar condition known as war edema, probably a nutritional disorder. Hulse [25] gave calcium salts

22. Müller, F.: Morbus brightii, Verhandl. d. deutsch. path. Gesellsch. **9**:64, 1905.

23. Aldrich, C. A., and McClure, W. B.: Intradermal Salt Solution Test, J. A. M. A. **82**:1425 (May 3) 1924.

24. Maase, C., and Zondek, H.: Das Kriegsoedem, Klin. Wchnschr. **54**:861, 1917.

25. Hulse, W.: Ueber den Einfluss der Kalksalze auf Hydrops und Nephritis, Centralbl. f. innere Med. **41**:441, 1920.

orally in large doses to patients with nephritis and noted a marked reduction in the edema. Many similar results have been reported with various explanations for the mode of action of calcium in this type of edema. It does not seem possible that the effect here can be attributed to the decrease in the permeability of the cell, which is probably of major importance in the control of inflammatory edema. The work of Skelton [17] is of great interest in this connection. He found, as stated previously, that after hemorrhage the tissues gave up fluid to the blood stream in relative proportion to the permeability of the capillaries in the respective organs as demonstrated by Starling; namely, the greatest relative loss occurred in the liver and the least in the skin and muscles. On the other hand, following the intravenous injection of a 2.4 per cent solution of calcium chloride, water was abstracted from all tissues, but the greatest loss occurred in the skin, where the permeability of the capillaries is relatively low. This fact, in the light of the observation by Schade [26] that the subcutaneous tissues possess an especial predilection for sodium chloride, seems to have a particular bearing on the problem of the therapeutic action of calcium in noninflammatory edema.

Fischer [6] stated that the edema which may follow the administration of sodium chloride or sodium bicarbonate is due to the replacement of calcium and magnesium in the tissues by sodium, for sodium proteins have a much greater affinity for water than calcium proteins. Frey [27] believed that the increased elimination of the fluid of edema by the kidneys may be explained by the fact that calcium is eliminated chiefly through the intestines, while the anion is excreted through the kidneys and draws with it the sodium ion. Whatever the true explanation may be, it is evident that the mutual physiologic antagonism of the sodium and the calcium ion has an important bearing on the problem of edema.

PRESENT INVESTIGATION

The present study was undertaken for the purpose of observing the effect of an increase in available circulating calcium on (1) the development of inflammatory edema and (2) the affinity of the tissue colloids for water.

METHODS

The increase in calcium was accomplished by the intramuscular administration of parathyroid extract.[28] In our previous studies, it was noted that from 15 to 20

26. Schade, H.: Untersuchungen zur Organfunktion des Bindegewebes, das Quellungsvermägen des Bindegewebes in der Männifaltigkeit seiner Erscheinungen, Ztschr. f. exper. Path. u. Therap. **14**:1, 1913.

27. Frey, W.: Die Einwirkung von Brom auf die Ausscheidung von Wasser und Kochsalz durch die Nieren, Verhandl. d. deutsch. Gesellsch. **33**:409, 1921.

28. The preparation used was Para-thor-mone supplied by Eli Lilly and Company through the courtesy of Dr. J. H. Warvel.

units of the hormone were usually sufficient to increase the amount of circulating calcium in the blood, the maximum effect being manifested in from eight to twelve hours after administration.[29] Determinations of serum calcium were made by the Clark-Collip modification of the method of Kramer and Tisdall.[30] Determinations of plasma calcium were made by the same method, powdered heparin being used as an anticoagulant.

The inflammatory reaction was produced by the blister method, used by Petersen[31] for the study of the permeability of the capillaries, with slight deviations from his suggested technic. A cantharides plaster, 1 cm. square (U. S. P. X.), was placed on the skin of the volar aspect of the forearm just below the elbow, at 6 a. m., and was removed four hours later. The interval of time between the removal of the plaster and the first suggestion of the formation of a blister was designated the blister time (B. T.). When sufficient serum had accumulated, it was withdrawn, and at the same time blood was taken from the vein. The protein content of the blister serum and blood serum was determined by the refractometric method, and the Reiss tables were used for the calculations, the exudate scale for the fluid of the blister and the serum scale for the blood serum. The ratio of blister serum protein to blood serum protein is termed, by Petersen, the permeability ratio (P. R.).

Estimations for blood calcium were made as stated, in several instances. One series of observations for control was made as outlined. At 6 p. m. of the same day, 20 units of parathyroid extract were injected intramuscularly; the same amount was administered at midnight, and the routine procedure and observations of the preceding day were repeated, the opposite forearm being used. By this procedure, the normal inflammatory reaction is compared with that occurring at the height of the effect of calcium in the blood and presumably in the tissues.

The affinity of the tissue colloids for water was determined by the test of McClure and Aldrich.[28] Two tenths of a cubic centimeter of physiologic solution of sodium chloride was injected intracutaneously at 8 a. m. on the volar aspect of the forearm, just below the elbow. The time of disappearance of the wheal was noted. Twenty units of parathyroid extract were then administered at 6 p. m. and again at midnight, and the test was repeated at 8 a. m. and at noon of the succeeding day.

TYPES OF CASES

The observations of the inflammatory reaction were made on patients with chronic pulmonary tuberculosis, of varying extent and activity. As Petersen[32] has found abnormal degrees of the permeability of the

29. Gordon, B. L., and Cantarow, Abraham: The Use of Parathyroid Extract in Hemorrhage, J. A. M. A. **88**:1301 (April 23) 1927. Cantarow, Abraham; Caven, W. R., and Gordon, B. L.: Changes in the Chemical and Physical Characteristics of the Blood Following the Administration of Parathyroid Hormone, Arch. Int. Med. **38**:509 (Oct.) 1926. Cantarow, Abraham; Dodek, S. M., and Gordon, B. L.: Calcium Studies in Jaundice, Arch. Int. Med. **40**:129 (Aug.) 1927.

30. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Calcium with a Suggested Modification, J. Biol. Chem. **63**:461 (March) 1925.

31. Petersen, W. F., and Willis, D. A. Capillary Permeability and the Inflammatory Index of the Skin in the Normal Person as Determined by the Blister, Arch. Int. Med. **5**:663 (Nov.) 1926.

32. Petersen, W. F.: The Permeability of the Skin Capillaries in Various Clinical Conditions, Arch. Int. Med. **1**:27 (Jan.) 1927.

capillaries in such patients, our observations obviously cannot be compared with those made on normal persons. However, the results obtained in the same person on successive days at the same hour and under the same conditions should be fairly comparable, the interval of time being too brief for any appreciable change in the clinical condition.

The patients on whom the intracutaneous test with a physiologic solution of sodium chloride was performed were suffering from cardiac or renal disease, some with and some without demonstrable edema.

TABLE 1.—*Results of Blister Test in Thirty Patients with Pulmonary Tuberculosis*

Case	Blister Time *		Blister Protein †		Serum Protein †		Permeability Ratio		Serum Calcium ‡		Plasma Calcium ‡	
	1§	2#	1	2	1	2	1	2	1	2	1	2
Group I (Far Advanced Tuberculosis)												
1	3:40	5:20	6.14	6.65	7.29	8.32	82.8	79.9	9.3	10.4	9.1	10.6
2	3:25	4:10	6.33	6.16	8.66	8.24	73.2	74.6	10.3	10.5	....	....
3	3:10	5:20	5.63	5.22	7.45	8.04	75.5	64.9	9.7	11.2	....	....
4	2:30	7:00	5.76	6.21	7.47	8.05	77.1	77.1	9.4	9.5	9.3	9.9
5	2:30	3:20	4.50	3.40	4.60	5.20	97.8	65.3	10.6	10.4	....	....
6	3:30	3:75	5.04	6.39	6.60	7.47	76.3	85.5	9.7	9.9	....	....
7	4:30	3:25	6.22	6.61	7.21	7.52	86.2	87.8	10.2	11.3	9.9	11.5
8	3:15	3:15	6.56	5.73	6.63	6.54	98.9	87.6	9.4	9.8	9.1	10.2
9	2:30	2:30	7.56	6.96	8.87	8.94	85.3	77.8	9.6	10.4	....	....
10	2:25	5:20	5.92	6.33	8.06	8.12	73.5	77.9	9.5	10.3	9.2	10.2
11	2:35	3:55	5.45	6.27	7.56	8.34	72.0	75.1	10.6	10.6	....	....
12	7:30	5:45	5.43	6.09	8.56	8.68	63.4	80.6	10.8	11.2	....	....
13	4:15	5:25	6.67	6.01	9.25	8.78	72.1	68.5	11.0	11.2	....	....
14	5:20	6:30	5.62	5.27	7.32	7.17	76.7	73.5	9.1	10.0	....	....
15	3:15	4:20	6.39	5.78	8.14	7.79	78.2	74.1	9.3	9.8	9.4	10.3
16	2:10	4:35	7.14	5.37	8.31	7.45	85.9	72.0	9.9	10.3	9.6	10.4
17	4:30	2:30	5.92	5.98	7.94	7.95	74.6	75.2	10.3	10.8	10.1	10.7
Average	3:35	4:35	6.31	5.96	7.64	7.79	79.8	76.3	9.9	11.0	9.4	10.4
Group II (Moderately Advanced Tuberculosis)												
18	2:25	2:30	8.54	8.03	10.79	9.96	79.2	83.1	10.4	10.9	10.0	10.8
19	4:35	2:30	6.98	6.29	8.45	7.74	90.8	81.3	10.8	11.2	....	....
20	3:10	2:55	5.52	5.54	7.27	7.09	75.9	78.2	11.1	11.4	....	....
21	3:30	4:35	6.40	6.17	9.69	7.76	66.1	79.5	9.7	9.9	....	....
22	4:15	5:35	5.90	6.76	8.34	8.30	70.7	81.5	10.2	11.0	10.0	11.0
Average	3:35	3:40	6.67	6.56	8.91	8.11	76.5	80.7	10.4	10.8	10.0	10.9
Group III (Inactive Tuberculosis)												
23	3:20	2:30	5.82	5.88	8.24	8.12	70.6	72.3	9.4	10.2	....	....
24	3:30	8:30	7.18	7.05	9.99	8.03	71.9	87.7	9.9	9.7	....	....
25	3:15	3:25	6.99	5.75	10.07	9.39	69.4	61.2	10.3	10.5	10.1	10.7
26	6:50	8:55	5.43	6.12	7.46	7.51	72.7	81.5	9.1	10.3	8.8	10.5
27	2:45	3:25	5.15	5.44	8.33	9.31	61.8	58.4	10.6	10.9	....	....
28	3:20	4:35	5.38	6.33	8.82	7.34	60.9	86.2	10.2	11.1	10.0	11.2
29	2:30	2:30	6.59	7.37	8.88	8.22	81.6	89.6	19.8	10.4	9.8	10.6
30	3:55	3:10	7.17	7.18	9.27	8.78	77.3	81.7	10.5	11.2	....	....
Average	3:40	4:35	6.20	6.39	8.88	8.34	70.7	77.3	9.9	10.5	9.7	10.7

* Blister time expressed in hours and minutes.
† Protein values expressed in Grams per hundred cubic centimeters.
‡ Calcium values expressed in milligrams per hundred cubic centimeters.
§ Before administration of parathyroid extract.
# After administration of parathyroid extract.

RESULTS OBTAINED

Blister Test.—The patients were divided into three groups according to the extent and activity of the pulmonary lesions, since Petersen found that variations in the permeability of the capillaries were dependent on the degree and type of the tuberculous process. The detailed results are presented in table 1.

Blister Time.—In the group of seventeen patients with far advanced active pulmonary tuberculosis, the blister time varied from two hours and ten minutes to seven hours and thirty minutes, the average being three hours and thirty-five

minutes. After the administration of parathyroid extract, the variation of time was from two hours and thirty minutes to seven hours, with an average of four hours and thirty-five minutes. In twelve patients, the blister time was prolonged; in two it remained constant, and in three it was diminished.

In the group of five patients with moderately advanced lesions, the time of appearance of the blister ranged from two hours and twenty-five minutes to four hours and thirty-five minutes, the average being three hours and thirty-five minutes. After the administration of parathyroid extract, the time varied between two hours and thirty minutes and five hours and fifty-five minutes, with an average of three hours and forty minutes. In three instances, the blister time was prolonged, and in two it was lessened.

In the eight patients with slight, inactive lesions, the blister time varied from two hours and thirty minutes to six hours and fifty minutes, averaging three hours and forty minutes; after the injection of parathyroid extract, the time ranged between two hours and thirty minutes and eight hours and fifty-five minutes, averaging four hours and thirty-five minutes. In five patients, the time was increased; in one it remained unchanged, and in two it was decreased.

Blister Proteins.—In group 1, there was one patient (case 5) with a marked secondary anemia and edema, and an extremely low serum protein content; in this case, the concentration of blister protein was 4.5 per cent before and 3.4 per cent after the administration of parathyroid extract.

If this case is excluded, concentration of blister protein in this group ranged between 5.04 and 7.56 per cent, the average being 6.31 per cent; after the injection of parathyroid extract, it varied from 5.22 to 6.99 per cent, and averaged 5.96 per cent. The concentration of protein was increased in eight cases and decreased in nine.

In group 2, the concentration of blister protein varied between 5.52 and 8.54 per cent averaging 6.67 per cent; after the administration of parathyroid extract, the variation was from 6.17 to 8.03 per cent, with an average of 6.56 per cent. The concentration was increased in two cases and decreased in three cases.

In group 3, the blister protein content varied from 5.15 to 7.18 per cent and averaged 6.2 per cent; after the administration of parathyroid extract, the variation was from 5.44 to 7.37 per cent, with an average of 6.39 per cent. The concentration was increased in six cases and decreased in two cases.

Serum Protein.—If case 5 in group 1 is excluded, the variation in the concentration of blood serum protein was between 6.6 and 9.25 per cent, the average being 7.64 per cent; after the administration of parathyroid extract, the concentration varied from 6.54 to 8.94 per cent and averaged 7.79 per cent. It was increased in eleven cases and diminished in six.

In group 2, the concentration ranged from 7.27 to 10.79 per cent, and averaged 8.91 per cent; after the administration of parathyroid extract it varied from 7.09 to 9.66 per cent, with an average of 8.11 per cent. The concentration was decreased in all five patients.

In group 3, the serum protein content ranged from 7.46 to 10.07 per cent, the average being 8.88 per cent; after the administration of parathyroid extract, the variation was between 7.34 and 9.39 per cent, averaging 8.34 per cent. An increased concentration occurred in three cases and a decrease in five cases.

Permeability Ratio.—The ratio of the concentration of blister protein to that of serum protein in group 1 varied from 63.4 to 97.8 per cent, the average being 79.8 per cent; after the administration of parathyroid extract, it varied from 64.9 to 87.8 per cent, averaging 76.3 per cent. It was increased in eight instances, stationary in one, and decreased in eight.

In group 2, the permeability ratio ranged from 66.1 to 90.8 per cent, averaging 76.5 per cent; after the administration of parathyroid extract, it varied from 78.2 to 83.1 per cent with an average of 80.7 per cent. It was increased in four cases and decreased in one case.

In group 3, the variation was between 60.9 and 81.6 per cent, and averaging 7.07 per cent; after the administration of parathyroid extract it ranged from 58.4 to 89.6 per cent, the average being 77.3 per cent. The ratio was increased in six patients and decreased in two.

Serum and Plasma Calcium.—The observations for calcium revealed nothing of unusual interest, except that the concentration of plasma calcium, which was slightly lower than that of serum calcium in most patients, after the administration of parathyroid extract was usually higher. The values for serum calcium were not appreciably increased in all cases, but, as shown by Hunter and Aub,[33] an increased availability and excretion of calcium may occur without a rise in blood calcium following the administration of parathyroid extract.

DISCUSSION OF RESULTS OF BLISTER TEST

If the time of appearance of the blister is an indication of the reaction of the tissues to irritation, it may be considered that in twenty of the thirty cases the rapidity of response to irritation was decreased after the administration of parathyroid extract; in three it was unchanged, and in seven it was increased. This would seem to support the conclusion that calcium tends to exert an inhibitory action on the production of inflammatory edema.

If, on the other hand, it is assumed that the permeability ratio, which is more or less directly dependent on the concentration of blister protein, is an index of the permeability of the capillaries, and, indirectly, of the inflammatory reaction, the results obtained are contradictory. The concentration of blister protein was increased in sixteen patients of this series and was decreased in fourteen, apparently indicating that the permeability of the capillaries in more than half of the cases was increased during the period of the increase in available tissue calcium. However, it is possible that the concentration of protein in the blister fluid may be dependent on factors other than the permeability to protein of the walls of the capillaries. Chief among these, perhaps, is their permeability to water and to crystalloids.

It is not unlikely that the concentration of blister protein depends on two variable factors: the amount of protein and the amount of water. If the former remains constant and the latter is diminished, the result will be an increased concentration of protein. Likewise, if both are diminished, but the water content is decreased relatively more than the protein, the same result will be obtained. A reduction in the amount of water in the blister may result from a diminution in the permeability

33. Hunter, D., and Aub, J. C.: Lead Studies 15. The Effect of Parathyroid Hormone upon the Excretion of Lead and of Calcium in Patients Suffering from Lead Poisoning, Quart. J. Med. **20**:136 (Jan.) 1927.

of the wall of the capillary to water or to crystalloids, the latter factor operating through the forces of osmosis. Furthermore, the inhibition of the inflammatory reaction by the excess of calcium may cause a diminution in the products of tissue disintegration accompanying the process; as a result of the subsequent relative reduction in osmotic pressure, less fluid will transude, although the amount of protein may be affected slightly.

It seems in these studies that the permeability ratio and the concentration of blister protein may not be true indexes of the permeability of the capillaries. There is one observation, however, which, though not of a definitely quantitative nature, may be considered significant. It was noted that after the administration of parathyroid extract, although the blister time may not have been prolonged or the concentration of protein decreased, the accumulation of fluid in the blister took place less rapidly than in the control test. In several instances, particularly in group 3, several hours elapsed after the first appearance of the inflammatory reaction before sufficient fluid had accumulated for determination of the protein content. This would seem to support the opinion that the increased concentration of protein is not due to an increase in the permeability of the capillaries but rather to a decreased transudation of water. It is not unlikely that the permeability to proteins is also diminished, but to a lesser degree, in the patients who show an increased concentration of blister protein after the administration of parathyroid extract. When the response in different persons is compared, the possibility of certain variable factors, such as the distribution of the capillaries circulation and the texture of the skin, should be recognized. This is true also of various transient physiologic and chemical changes which may occur in the same person. The data, therefore, should be considered critically.

INTRADERMAL TEST WITH PHYSIOLOGIC SOLUTION OF SODIUM CHLORIDE

Observations were made on twenty patients, as described before, suffering with cardiac or renal disease. The observations are detailed in table 2.

In eighteen patients, the level of the serum calcium ranged from 9.1 to 10.2 mg. per hundred cubic centimeters, and the plasma calcium from 8.8 to 9.9 mg. per hundred cubic centimeters. In cases 10 and 15 the patients had far advanced cardiorenal disease with general anasarca, the serum calcium values being 8.4 and 8.5 mg. per hundred cubic centimeters, and the plasma calcium values, 7.9 and 8.3 mg. per hundred cubic centimeters, respectively. In every instance the level of the blood calcium was raised following the administration of parathyroid extract.

The duration of the wheal corresponded fairly closely to the degree of edema present. The time varied from twenty-five (case 15) to eighty minutes. In seven cases, the injected fluid disappeared in less than sixty minutes, the low limit of normal, and in these patients there was definitely palpable edema. In three cases in which the disappearance time was sixty minutes, there was slight edema of the ankles.

In every instance, twelve hours after the second administration of parathyroid extract, there was a prolongation of the disappearance time of the injected fluid, varying from five to forty minutes and averaging twenty-four and one-half minutes. The results were particularly striking in that in all but one case the duration of the wheal was well above

TABLE 2.—*Results of Intracutaneous Test with Physiologic Solution of Sodium Chloride before, and Eight and Twelve Hours after the Administration of Parathyroid Extract in Twenty Patients with Cardiovascular Renal Disease*

		Control			Eight Hours			Twelve Hours		
			Calcium *			Calcium *			Calcium *	
Case	Edema	Minutes	Serum	Plasma	Minutes	Serum	Plasma	Minutes	Serum	Plasma
1	None	65	10.2	9.8	80	....	....	85	....	....
2	Slight, ankles	60	9.8	9.5	65	....	....	85	....	....
3	Slight	55	9.2	8.9	65	....	....	80	....	....
4	None	70	9.7	9.3	70	....	....	85	....	....
5	None	75	9.1	8.8	90	11.1	10.8	110	10.3	10.6
6	Ankles	60	10.2	9.9	75	12	12.2	65	11	10.8
7	None	70	10	9.5	80	12.2	11.9	100	11.1	11.4
8	None	65	9.3	9.1	80	12	12.1	80	10	10.2
9	None	80	10.1	9.9	85	12.3	12.5	105	11.2	11.5
10	Legs, face, ascites	45	8.4	7.9	..	....	....	70	10.1	10.4
11	None	75	9.6	9.2	..	....	....	95	....	....
12	Ankles, legs	55	9.7	9.3	..	....	....	80	....	....
13	Slight, ankles	60	9.4	8.8	..	....	....	80	....	....
14	Legs, ascites	50	...	...	..	....	....	85	....	....
15	General anasarca	25	8.5	8.3	..	....	....	40	9.2	9
16	None	65	...	...	..	....	....	90	....	....
17	Ankles	55	...	...	..	....	....	85	....	....
18	Legs	50	...	...	..	....	....	80	....	....
19	None	60	...	...	..	....	....	100	....	....
20	None	70	...	...	..	....	....	105	....	....

* Calcium values are expressed in milligrams per hundred cubic centimeters.

the normal low limit, and in eleven it was above the high limit. In the single case in which the time remained below normal (case 15), it was increased from twenty-five to forty minutes. This patient was the only one of the series with demonstrable edema of the arms, the test being performed, therefore, in definitely edematous tissues.

In nine patients, the test was also performed eight hours following the injection of parathyroid extract (table 2). The disappearance time was found to be increased in all but one (case 4), the prolongation in the other eight cases varying from five to fifteen minutes and averaging eleven minutes. Determinations made eight and twelve hours after the administration of parathyroid extract showed that the serum and plasma calcium were higher at the end of eight hours than after twelve hours. In every case but two (cases 6 and 8), the disappearance time was

distinctly longer after twelve hours than after eight hours. This serves to support the hypothesis stated previously, that the production of a calcium effect is dependent not entirely on an elevation of total circulating calcium, but on an increase in the availability of calcium. This may be expressed as an increase in the ratio of the diffusible or the ionized fractions to the total calcium.

In this group of patients, the edema was of the noninflammatory type. It would appear that as a result of the increase in available calcium following the administration of parathyroid extract, the capacity of hydration of the tissue colloids was diminished.

Some time after this work had been completed, McCann [34] reported three cases of nephritis with edema in which he had used parathyroid extract in therapeutic dosage. Marked and prolonged diuresis occurred in each case, with diminution in the edema and increased urinary excretion of sodium chloride. These observations seem to coincide with the results of the intradermal test with physiologic solution of sodium chloride, mentioned previously.

CONCLUSIONS

It appears that if the blister test is to be used to study the permeability of the walls of the capillaries, perhaps more reliance should be placed on the blister time and the rate of accumulation of fluid in the blister than on the concentration of protein in the blister fluid or the permeability ratio.

Utilizing the blister test as a means of studying inflammatory edema, it would appear that an increase in available circulating calcium effected by the administration of parathyroid extract exerts a slight but rather constant and definite inhibitory effect on the inflammatory process. This effect may be due to a decrease in the permeability of the capillaries or to a relatively decreased osmotic pressure in the irritated tissues. If the action is chiefly one of diminished permeability, the passage of water through the walls of the capillaries is inhibited in many cases to a greater degree than is the passage of proteins. It is possible that the result is due to a combined effect on osmosis and permeability.

The observations of the intradermal test with physiologic solution of sodium chloride suggest that calcium decreases the affinity of the tissue colloids for water. The exact mode of action is not entirely clear. It is not unlikely that the calcium replaces the sodium which has accumulated in the tissues, the reduction in edema being due to the fact, as mentioned by Fischer,[6] that calcium protein compounds have a lower capacity for hydration than have sodium protein compounds.

34. McCann, W. S.: Diuretic Action of Parathyroid Extract-Collip in Certain Edematous Patients, J. A. M. A. **90**:249 (Jan. 28) 1928.

SUMMARY

1. The principal factor underlying the production of inflammatory edema is probably increased permeability of the walls of the capillaries. It appears that the administration of calcium exerts an inhibitory influence in this type of edema perhaps as the result of the diminution in the permeability of the capillaries.

2. The chief factor underlying the development of noninflammatory edema is probably an increased capacity of the tissue colloids for hydration. Retention of sodium is an important factor, but whether primary or secondary, is uncertain.

3. Inflammatory edema was studied by the blister method, the observations being repeated at the height of the effect of calcium produced by the intramuscular administration of parathyroid extract.

4. The increase of calcium seemed to have a slight but definite inhibitory effect on inflammatory edema.

5. The affinity of the tissue colloids for water was studied by the intradermal injection of physiologic solution of sodium chloride, the test of McClure and Aldrich, in a group of twenty patients with cardiac or renal disease, some with and some without edema. The test, repeated after the administration of parathyroid extract, indicated a marked decrease in the capacity of the tissues for hydration, which accompanied the increase in available circulating calcium.

Book Reviews

SINDROMI NEURO-IPOFISARIE. By G. DI GUGLIELMO. Price, 50 Lire. Pp. 311. Milano: S. A. Instituto editoriale scientifico, 1928.

In the introduction the author recalls the history of our conceptions concerning the relationship of the pituitary gland to the hypothalamus since Marie's description of acromegaly, noting the emphasis on the hypophysis, following Froehlich, the reaction led by Camus and Roussy, and finally the conception of Perde that there is a symbiotic collaboration of neurohypophysis and hypothalamus. It should be noted from the outset that the author, although professing to treat of syndromes due to lesions of the pars posterior hypothalamus, inserts also many conditions due purely to lesions of the anterior lobe, such as the malady of Simmonds.

The first chapter is concerned with anatomy. The description of the hypothalamus follows Ramirez-Corria (reference not given), le Grand and Kary. The author seems not to be familiar with the fundamental work of Spiegel and Zweig and of Greving, not to mention the work of Malone in this country. His description of the hypophysis occupies only half a page. The chapter closes with an account of the tractus supraoptico-hypophysios.

The second chapter dealing with the physiology and physiopathology is more adequate and gives a fairly complete summary of experimental work on the relationship of pars posterior and hypothalamus to such functions as regulation of the metabolism of salt, water, carbohydrates, and fat, growth, sexual activity, sleep and heat production. He concludes that anatomic connections and functional correlations exist between the hypothalamus and pars nervosa in such manner that they constitute a unitary system, each one influencing the other, the pars nervosa exercising its influence by its secretion and the hypothalamus by nervous impulses. Disturbances due to lesions of this system he proposes to call neurohypophysial syndromes.

In the etiology of these syndromes, tumors occupy a prominent place and the author lists adenomas, sarcomas, gliomas, fibromas, keratomas, carcinomas and hypophyseal duct tumors. In this list he follows the older pathologists, since carcinomas, fibromas and gliomas are rare and sarcomas are never found in the hypophysis. Other causes listed are hemorrhage, thrombosis, embolism, cysts, trauma and foreign bodies.

The author essays a classification of neurohypophysial syndromes as follows:

1. Syndromes due to altered metabolism of fat.
   a. Defect syndromes (malady of Simmonds).
   b. Excess syndrome (obesity, adiposogenital dystrophy).
   c. Mixed syndromes (progressive lipodystrophy).
2. Syndromes due to altered water-salt metabolism.
   a. Defect syndromes (oliguria).
   b. Excess syndromes (polyuria, diabetes insipidus).
3. Syndromes due to altered growth.
   a. Defect syndromes (nanism, infantilism).
   b. Excess syndromes (gigantism, acromegaly, pubertas praecox).
4. Syndromes due to altered carbohydrate metabolism.
   a. Diabetes mellitus (atypical forms).

This classification is misleading, for obesity and adiposogenital dystrophy are deficiency syndromes, and the opposite of the malady of Simmonds is to be sought in acromegaly, just as gigantism is the opposite of infantilism. Most writers would consider the obesity to be the opposite of diabetes mellitus. Moreover, the malady of Simmonds, nanism, gigantism and acromegaly do not pertain to the neurohypophysis-hypothalamic system but rather to the pars anterior.

The author points out that the diagnosis of these syndromes is aided considerably by ocular defects and radiography. He is opposed to surgical treatment, more favorably inclined toward cautious radiotherapy and believes in the efficacy of extracts of the anterior lobe which have been shown experimentally to be totally inactive.

The second part of the monograph deals with the deficient syndromes of his classification, each being considered separately with clinical examples. He begins with Simmond's disease, which he defines as a morbid entity characterized clinically by denutrition, adynamia, apathy, somnolence, precocious senility, falling of hair and teeth, dryness of skin, amenorrhea, atrophy of internal and external genitalia, low blood pressure, eosinophilia and microsplanchnia; and anatomically by atrophy of the anterior lobe of the hypophysis from various causes, with probably participation of certain diencephalic centers. The history, symptoms, course, diagnosis, pathologic anatomy, etiology, pathogenesis, prognosis and therapy are discussed. The constant efficacy of the anterior lobe in the treatment is emphasized, which is interesting when one considers that such preparations are totally inactive in experimental animals. A clinical case is given with a complicated syndrome including diabetes insipidus. The patient was a prostitute, aged 29, syphilitic, who seems to have improved remarkably following the administration of pituglandol.

Adiposogenital dystrophy is treated in the same way with three cases as illustrations: the first a tumor of the hypophysis, the second too briefly recorded to allow of a diagnosis and the third probably a tumor of Rathke's pouch. All are clinical cases without verification at necropsy or operation.

In the section dealing with progressive lipodystrophy the author has great difficulty in bringing the neurohypophysial system into its etiology. This section is also illustrated by a clinical case.

The discussion of diabetes insipidus is more adequate, as the author is here on firmer ground. He seems on the whole inclined to accept the conclusions of Zadek that destruction of the hypothalamus is responsible for the reduction in power of the tissues to fix water, while the pars intermedia and peduncle of the hypophysis are responsible for hypochloremic diabetes insipidus. He does not make any attempt to reconcile these conclusions with the fact that experimental removal of the pars intermedia and pars nervosa does not result in polyuria. Seven cases of diabetes insipidus are discussed. Necropsies were obtained in two of them; in the second of these the patient had also a marked glycosuria. In the first case, examination of the hypophysis showed the presence of atypical cells in the anterior lobe, of basophil cells in the posterior lobe and of an unusual amount of colloid in the neurohypophysis. Nothing is said of the hypothalamus. In the second case, examination of the hypophysis showed a considerable area of the anterior lobe to be transformed into connective tissue and thickening of the capsule. The pancreas is said to have been normal. Again nothing is said of an examination of the hypothalamus.

The monograph closes with a chapter on syndromes of altered somatic development, although the author states that these cases are not due to lesions of the neurohypophysial system. A clinical case of macrogenitosomia praecox is given without evidence of involvement of the hypophysial region. After discussing the different classifications proposed for these developmental disorders three cases of hypophysial dysgenopathy are discussed, two with necropsy. In the first case there is no clinical history. The necropsy disclosed a congenitally defective heart. In the report of the necropsy is the bare statement that the "infantilism was very manifest." In the anterior lobe of the hypophysis a large cyst was found, and there was marked hyperemia of the remaining glandular tissue. The second observation concerns a man, aged 19, with endocarditis causing mitral, tricuspid and aortic insufficiency. In infancy he had had a febrile disorder which resulted in a deformity of the right foot. He was underdeveloped. Examination of the hypophysis revealed a large cyst between the anterior and the posterior lobes.

The monograph as a whole adds nothing to our knowledge of the pathophysiology of either the hypophysis or the hypothalamus.

BLOOD AND URINE CHEMISTRY. By R. B. H. GRADWOHL and IDA E. GRADWOHL. St. Louis: C. V. Mosby Company, 1928.

The first twenty chapters of this volume consist of a clear, concise and thorough description of the methods ordinarily used in the chemical analysis of blood and urine with special emphasis laid on colorimetric procedures. It is to be regretted that mention is not made of the technic of estimations of sodium or potassium in the blood or of total bases, of blood hemoglobin, blood sulphates and fractional determination of plasma proteins. The only method testing chlorides in the plasma is the Volhard-Arnold method, and none of the recent p_H methods are even mentioned. Throughout the book there are lengthy quotations from articles in the literature, giving the appearance of an exhaustive review but, unfortunately, they are not always up-to-date, although in some places they are. For example, in the discussion of the dextrose threshold and dextrose tolerance test, mention is not made of the vast amount of careful work in Faber's clinic, and in the chapter on nephritis there is nothing quoted more recent than 1917 or 1918, except in the discussion of the toxemias of pregnancy.

The arrangement is sometimes puzzling. For example, the whole subject of blood calcium and a long review of the chemical changes in the blood in intestinal obstruction are given in the chapter on nephritis, whereas nephrosis is briefly mentioned. The chapters in which gout and renal glycosuria are discussed are much better.

The last part of the book consists of a review of basal metabolism most of which is quoted partly from McLeod's book on physiology and biochemistry in modern medicine, Boothby and Sandeford's laboratory manual on basal metabolism and the booklet of description of technic which comes with the Benedict-Roth apparatus.

The book is well printed and excellently illustrated. It is to be hoped that the second edition will be a little more even in its subject matter and up-to-dateness.

OPERATIVE SURGERY. By J. SHELTON HORSLEY. Third edition. Price, $15. St. Louis: C. V. Mosby Company, 1928.

Since the last edition in 1924, much material has been added to this book, one of the standard works on operative surgery, so that it now embraces nearly 900 pages. It is well illustrated with drawings, mostly original, and is beautifully printed and bound.

The new material deals especially with the heart and lungs, as well as with several new intracranial operations, and makes up an admirable summary of the advances in surgery during the past few years.

While matters of diagnosis and nonsurgical aspects of disease are of course excluded, the work contains summaries of many of the newer contributions to the basic sciences underlying our surgery, such as the work of Gye, Barnard, and Bell on carcinoma, and a great deal of modern experimental surgery is summarized in the proper places when it has bearing on clinical problems.

The text is clear, and by frequent reference to the illustrations a good idea may be gained of the technic of each particular operation. The choice of material has been on the whole wisely made, the commoner operations receiving the most attention. Bone surgery is perhaps somewhat slighted in view of the increasing specialism of this sort of work and the many volumes by other authors devoted to it.

I would say that the only criticism to make is the undue amount of space of nearly 100 pages given to the discussion of blood vessel surgery, including transfusions of blood. In the latter field especially, many of the statements regarding the advantages of the various methods might well be argued. Certainly, Crile's method of direct vascular anastomosis is rarely carried out today.

On the whole, the reviewer feels convinced that this work is the most valuable treatise on the subject today.

PHARMACOTHERAPEUTICS, MATERIA MEDICA AND DRUG ACTION. By S. SOLIS-COHEN and T. S. GITHENS. Price, $15. Pp. 2009. New York: D. Appleton & Company, 1928.

This work is a noteworthy addition to the available texts on therapeutics and pharmacology, being the most comprehensive and complete work on drug therapeutics in the English language. The authors present pharmacologic justification for the use of the drugs used in modern therapeutics. Pharmacology is well covered, and in many respects this text may even be considered as a textbook of pharmacology, though it is written from the clinical standpoint using pharmacology as a basis for therapeutic uses. In some respects, the authors are conservative, whereas in others they are apparently overoptimistic in that some of their conclusions are drawn from evidences which could scarcely be considered as adequate.

The book is exceedingly valuable and can be read with profit by both the clinician and the laboratory man. To the research man the book is both valuable and disappointing since authors' names are generously cited, but literary references are not given.

THE TREATMENT OF DIABETES MELLITUS. By ELLIOTT P. JOSLIN. Fourth edition. Price, $9. Pp. 998. Philadelphia: Lea & Febiger, 1928.

In 1916, the first edition of Joslin's "Diabetes" appeared in a large volume of 440 pages. The new fourth edition, arriving just twelve years later, contains 998 pages. Such are the complications of modern medicine that one student working intensively at one disease can produce such an encyclopedic volume. The work represents the characteristic Joslin trait of painstaking study of his own work and of careful inquiry into the work of others, all with one end in view—improvement in the treatment of the diabetic patient. The reviewer finds it difficult to attempt a detailed comment; the book contains so much that one can open it at random and receive almost at a glance an impetus to review his own cases of diabetes. This volume is so full of information on so many aspects of the disease other than treatment that it is today the outstanding practical treatise on diabetes mellitus.

LOBAR PNEUMONIA. By L. R. SANTE. Price, $3. New York: Paul B. Hoeber, 1928.

Sante's brief monograph records his experience in the x-ray examination of patients with lobar pneumonia at the St. Louis City Hospitals and elsewhere in St. Louis.

A reader not conversant with the technical difficulties of irradiation of patients with pneumonia and the bewildering clinical problem of differential diagnosis of x-ray films of the chest in pneumonia would find in this book little to suggest such difficulties.

Few clinical workers will deny the immense value of the roentgen ray in establishing an essentially normal condition of the lung when disease is feared or in outlining roughly the anatomic distribution of lesions when lesions exist; however, the time has not yet arrived when unsupported x-ray evidence will always answer the question, "Is it consolidation or is it fluid?"

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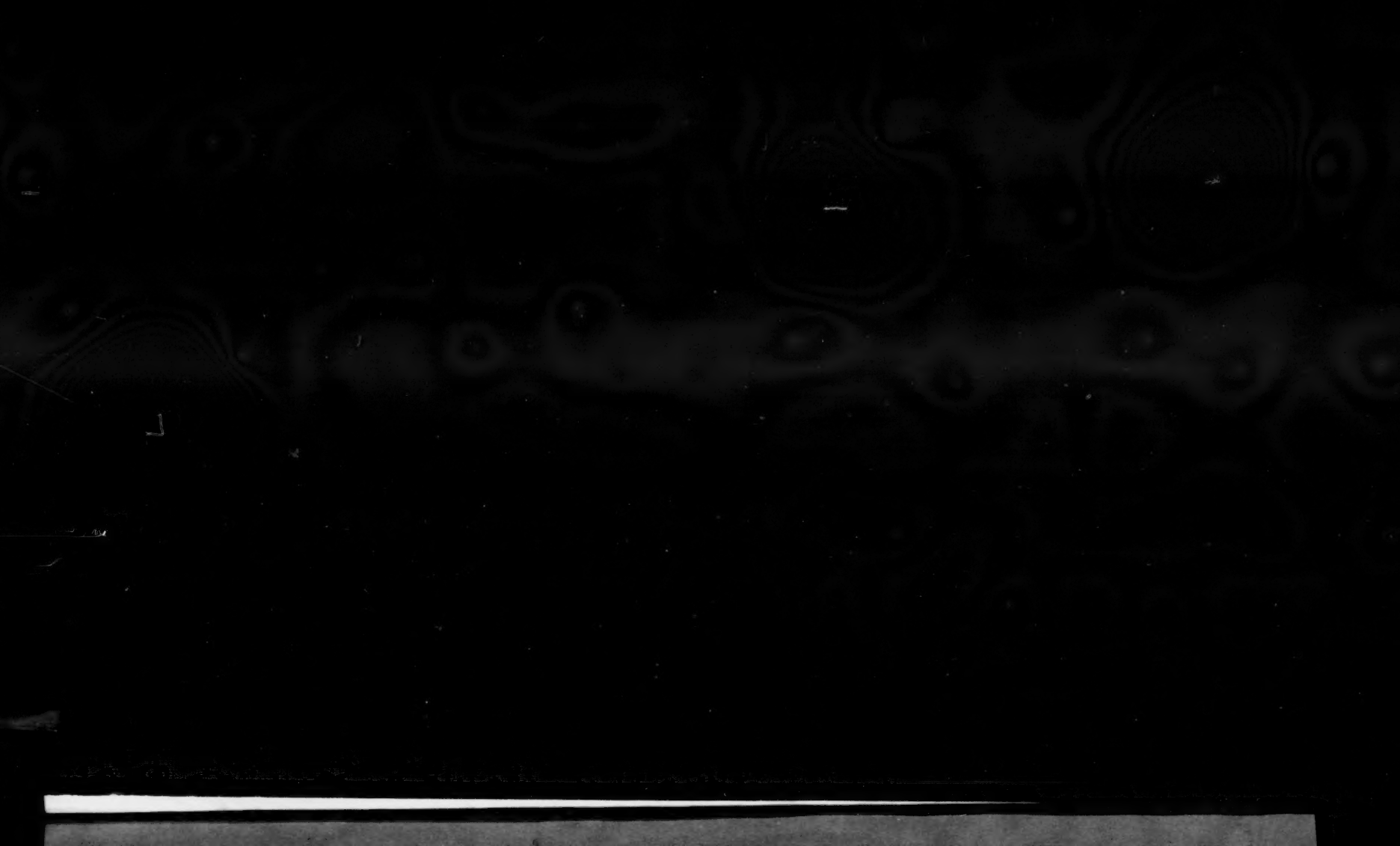
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